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This number of the *Journal*
is dedicated
with respect and affection
to
CHARLES SCOTT SHERRINGTON, O.M.
who introduced the concept of
The Synapse,
as well as the word itself,
into the literature of neurophysiology

PREFATORY NOTE

Symposium on the Synapse

THE PAPERS contained in the September number of the *Journal of Neurophysiology* formed the subject matter of a Symposium on the Synapse held at Toronto, April 29, 1939, during the Annual Meeting of the American Physiological Society.

THE EDITORS

AXONS AS SAMPLES OF NERVOUS TISSUE*

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Laboratories of The Rockefeller Institute for Medical Research, New York

(Received for publication May 27, 1939)

THE FIRST question that would naturally be asked about the synapse is: What is the nature of the material coming in contact at its borders? The answer to the question must come from a direct study of the synaptic region of the neuron, and for that reason it is a difficult one to obtain. As an introduction to the problem, attention has been directed to properties of the axon, because of the expectation that the events which take place in those parts of the neuron entering into the synapse may resemble, qualitatively at least, events taking place in other parts of the neuron. If it could be shown that the expectation has a foundation in fact, to the extent to which it holds, axon physiology could be transferred directly to synapse physiology.

As is well-known, axons are not all alike. This fact in itself is helpful for our present purpose, as samples of different kinds of nervous tissue are presented for review. The common features among the characteristics of these samples may be taken to give an indication of the qualities that are shared generally by nervous structures, and the mode of variation of the characteristics may be taken to give an indication of the directions in which differences are to be anticipated.

I want to mention with the greatest possible brevity the properties of the action in nerve fibers that appear to have application to synaptic conduction. The illustrations which will be cited are typical for the three kinds of nerve fibers, A, B, and C. The designation A refers to the somatic myelinated fibers; B to the autonomic myelinated fibers, that is, the group originally described by Bishop and Heinbecker as B_2; and C to the unmyelinated fibers.

Action in all fibers starts with a spike. The only difference between one type of fiber and another in this regard is in the duration (Fig. 1). The A spike stands at one end of the range with a duration of 0.4 msec. and the C spike at the other end with a duration of a little over 2 msec.

The spike is followed by an after-potential much smaller in size and much greater in duration. After-potentials vary as to form, size, and duration, depending upon the kind of fiber (Fig. 2). The complete sequence is a negative after-potential followed by a positive potential. It is found clearly developed in A and C fibers. In B fibers the negative after-potential is vestigial in single responses, and it appears only after certain forms of activity and after special experimental procedures. When records are prepared at low amplification, as in Fig. 2, so that both the spike and the after-potential are visible in the same tracing, the variation in the size of the positive after-potential

* Symposium on the Synapse, Meeting of The American Physiological Society, Toronto, April 29, 1939.

at once strikes the eye. The positive after-potential is readily visible in the B fibers and somewhat less so in the C fibers, while in the A fibers the configuration that appears so clearly at higher amplification is all but indistinguishable.

Spikes are generally considered to be the message carriers; that is, some agent, physical or chemical, directly under the control of the spike process

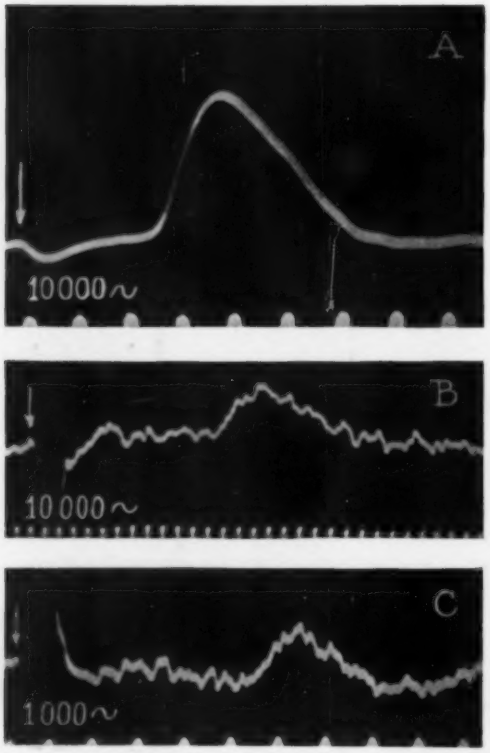

FIG. 1. Spikes of A, B and C fibers; A, potential from a single large fiber of a dorsal spinal root of the cat; B, from the cervical sympathetic nerve of the rabbit, threshold response, possibly not a single fiber (spike 25 μv.); C, from the splenic nerve of the cat, threshold response (spike 20 μv.). The fine oscillations are occasioned by the noise level.

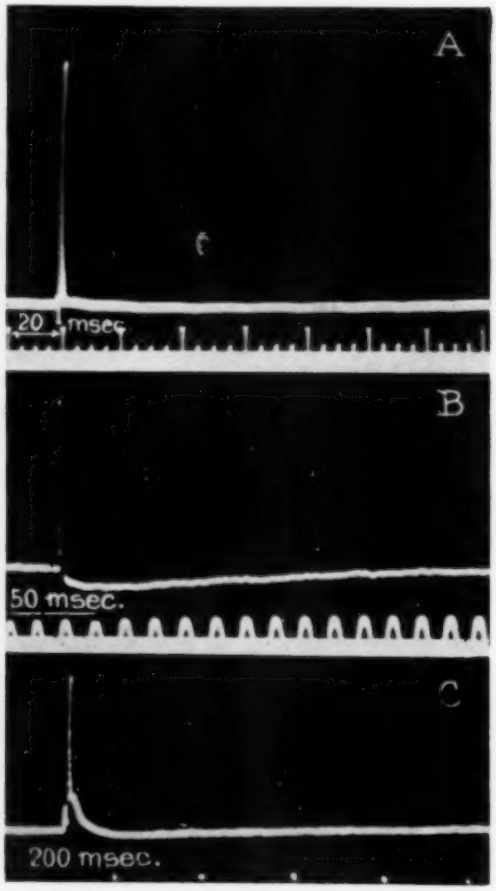

FIG. 2. Action potentials of A, B and C fibers (cat): A, from the phrenic nerve; B and C from hypogastric nerves.

is held responsible for the mediation of transmission. The view that spikes serve this function is well founded, but other views have not thereby been excluded. The contemporary literature contains arguments for impulse initiation by slow potentials as well.[1]

One point is certain with respect to the after-potentials; they influence the level of excitability. During the negative after-potential the fibers are supernormal, and during the positive potential they are subnormal (Fig. 3).

Furthermore, when the configuration of the after-potential is altered, as it easily can be in a completely reversible manner by a wide variety of conditions or of states of activity, the excitability curve is altered in parallel with it. The same parallelism that holds between excitability and after-potential form for the various states of a single type of fiber, also obtains for the variation of the after-potential as it appears in the different types. The course

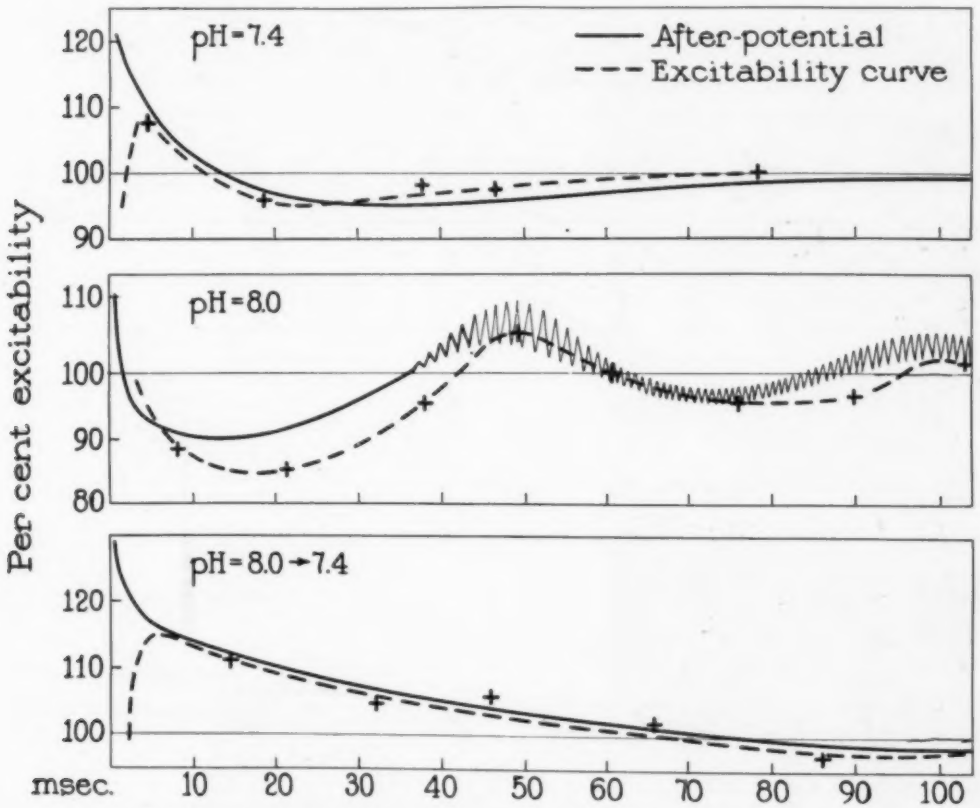

FIG. 3. Relation between the excitability curves and the after-potentials in A fibers (phrenic nerve of the cat) Lehmann[5] (1937). The three parts show from above downward the normal condition, alkalinity resulting from removal of CO_2 from the atmosphere, and an early stage following restoration of CO_2 before the normal steady state is reached. The ordinates give the reciprocal of the threshold for excitation in percentage of the resting threshold.

of the excitability curves following a single response is plotted for the three types of fibers in Fig. 4. The curve for the C fibers[6] resembles that for the A fibers but for the fact that it is much more drawn out in time. As would be expected, the B fiber curve differs from both of them. In keeping with the absence of negative after-potential, there is no early supernormal period.

When a nerve is tetanized, the positive after-potential following the last spike in the train is larger than one following an isolated spike. The manner in which the growth takes place in A fibers is shown in Fig. 5, and an analo-

gous process occurs in the other types. If the tetanization is severe enough, the positive after-potential at the end of the tetanus, which corresponds to the one seen after a single response, is followed by a second positive potential. Unlike the first, the second potential increases in duration as well as size as the length of the tetanus and particularly the frequency of the tetanus are increased. The end result is the same in all fibers (Fig. 6)—an after-potential yielding records characterized by an initial sharp notch followed by a long

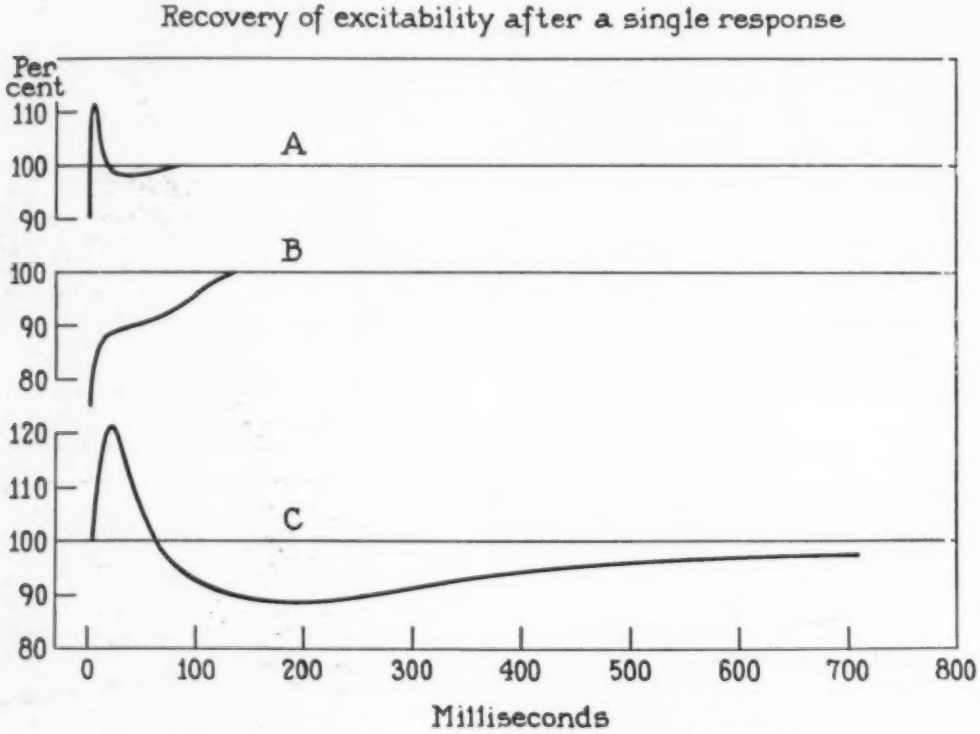

FIG. 4. Excitability curves of A, B and C fibers shown for comparison on the same time scale.

shallow trough. But for the time scale and the size of the potential in comparison with the height of the spike, the after-potential following a tetanus of C fibers resembles greatly that recorded after a tetanus of A fibers.

The excitability of a nerve following a tetanus is exactly as would be predicted from the after-potentials: the longer the duration and the higher the frequency of the tetanus, the greater and more prolonged the ensuing subnormality. Some of the early stages in the development of this subnormality are shown in Fig. 7. Among other things, the curves show that the supernormal period is a phenomenon restricted to very mild activity and that supernormality following the cessation of the tetani responsible for message transmission could hardly occur.

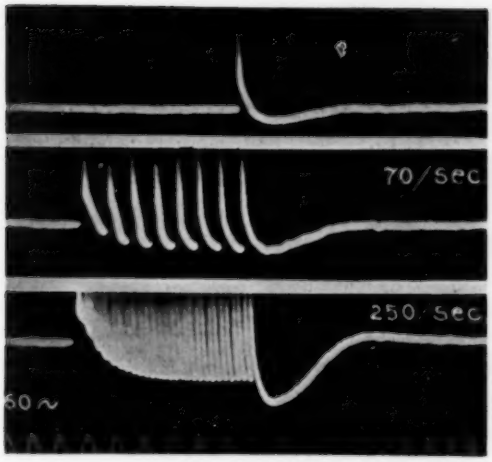

FIG. 5. Increase in the positive after-potential produced by a short tetanus (A fibers, phrenic nerve of the cat, 37°C., 5 per cent CO_2 in O_2). The records start with the negative after-potential. The spikes would extend far above the tops of the records. Changes in the negative after-potential are also to be noted.

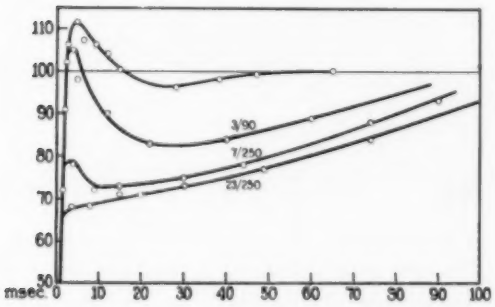

FIG. 7. Recovery of the saphenous nerve *in situ*, decerebrated cat. The ordinates give the reciprocal of the threshold strength of stimulation in percentage of the resting threshold; the abscissae, the time after the end of the conditioning excitation. In the course of the experiment conditioning was changed from a single action to a tetanus. 3/90 means conditioning by 3 shocks at 90 per sec. (Gasser and Grundfest[4] 1936).

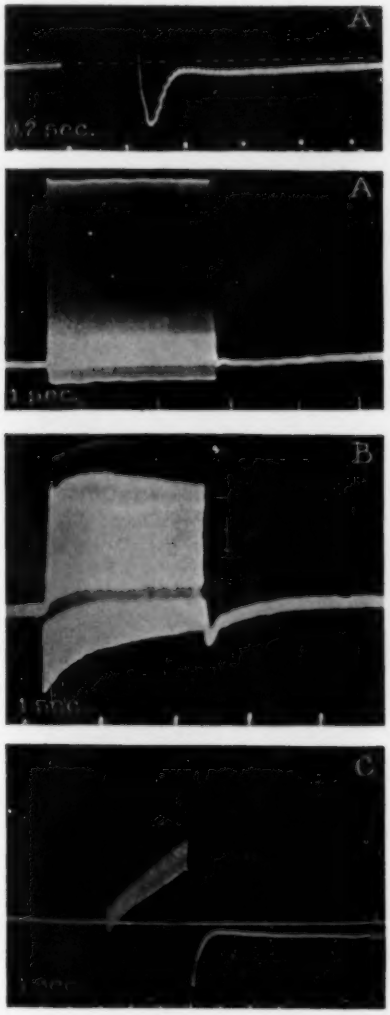

FIG. 6. Tetani of A, B and C fibers: A (lower), from the phrenic nerve of the cat; B and C, from the hypogastric nerve of the cat. The tops of the A and C spikes are at the tops of the records, the tops of the B spikes at the tops of the heavy white lines. The form of the A after-potential can best be seen in the upper record taken with higher amplification and with a faster sweep than in the record below it.

So much for the axon. Now, do any analogous phenomena occur at the dendritic end of the neuron? Numerous bits of information indicate that they do, but it would be impossible to summarize them within the compass

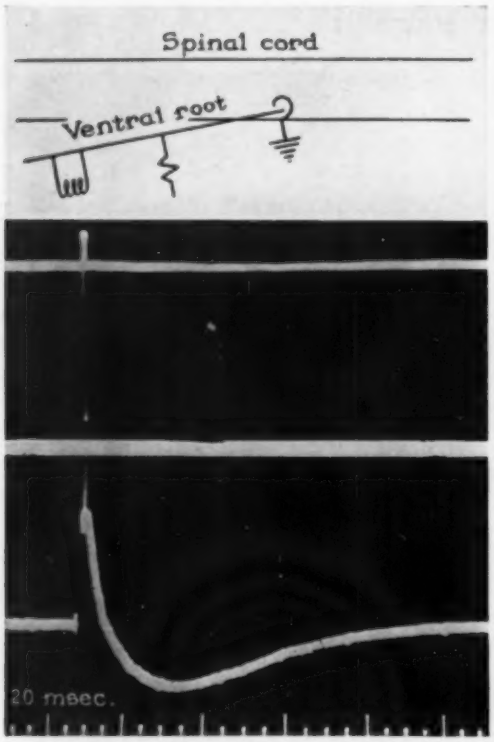

FIG. 8. Records obtained with the leads shown in the diagram when a single volley was backfired into the spinal cord of a cat through a motor root. (Dial narcosis). The lower record is at approximately 100 times the amplification of the upper. Control observations showed that the potential contributed by the spinal cord was not caused by a spread of the stimulating current to central structures but, as held by Eccles and Pritchard, was evoked by the antidromic volley itself.

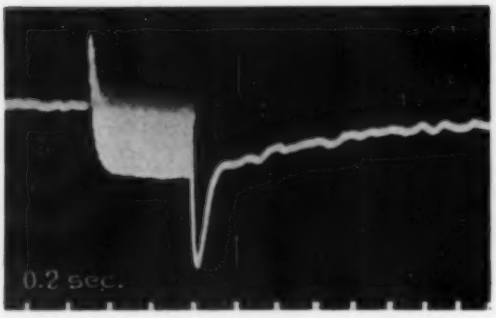

FIG. 9. Leads as in Fig. 8. The record shows the potential evoked by an antidromic tetanus. (Dial narcosis.)

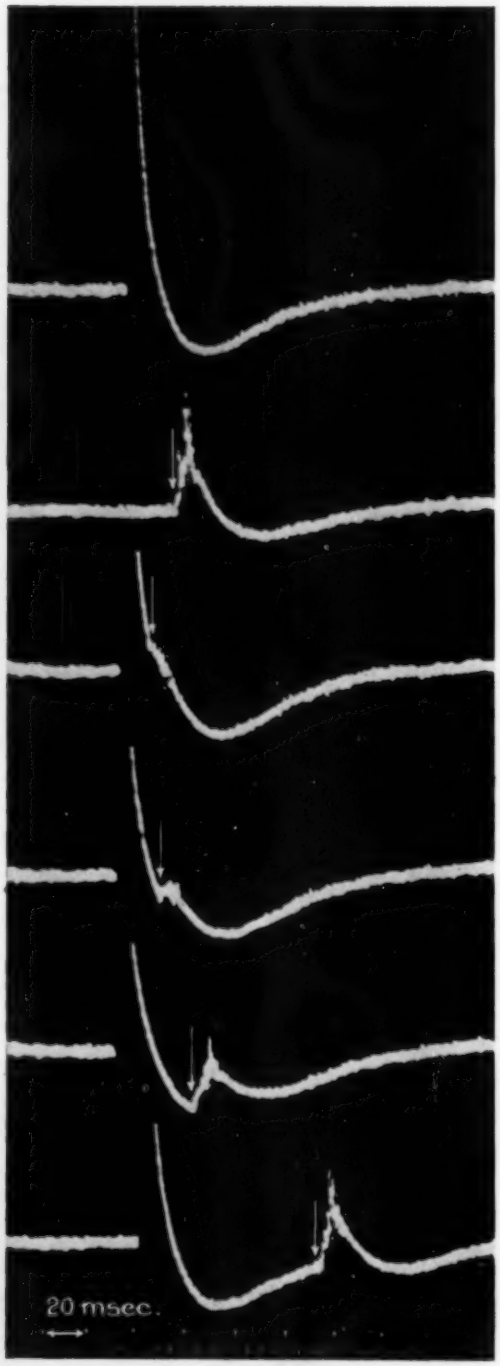

FIG. 10. Leads as in Fig. 8, except that both electrodes were on the root, the central electrode about 2 mm. from the cord. (Dial narcosis.) The upper record was produced by a single antidromic volley. The second record was produced by a single volley entering the cord through the dorsal root of the same segment. The potential was occasioned chiefly by the motor reflex discharge. The remaining records show the conditioning effect of the antidromic volley upon the size of the reflex. Arrows indicate the moment of stimulation of the dorsal root.

of the present discussion. Only one point will be brought forward, that is, subnormality in motor neurons. That motor neurons have a subnormal period similar to that of the axon first became apparent in considerations involving the interpretation of the silent period following a reflex discharge (Gasser,[3] p. 199). The arguments adduced in support of the association of the two events can now be passed over, however, because new and more direct evidence has become available from the experiments of Eccles and Pritchard,[2] in which a study was made of the effects of backfiring an antidromic volley of impulses into the spinal cord by way of a motor root.

Figure 8 was prepared in the course of repetition of one of Eccles and Pritchard's experiments. The first sacral motor root at its exit from the spinal cord was severed and stimulating electrodes were placed near the cut end. A single volley was backfired into the cord and the action potential led off from the side of the root and from the cord adjacent to the point of emergence of the root. The diphasic spike obtained at low amplification helps in the clarification of the potential picture obtained at high amplification. The latter has the appearance of a monophasic action potential of an A fiber (spike upward) as observed at high amplification (Fig. 5). Evidently, as concluded by Eccles and Pritchard, the form of the action potential of the intramedullary portion of the neuron is being revealed. If the cord is asphyxiated, the potential rapidly disappears and there is left only the after-potential of the root fibers. Under the conditions of the experiment, the centrally contributed portion of the potential so dominates the contribution from the root that in the algebraic summation of the two in the records, the configuration of the centrally produced potential is not obscured.

The duration and form of the central positive potential evoked by an antidromic volley are the same as the duration and form of the positive after-potential in a single action of A fibers. After a tetanus the similarity between the two potentials still holds. When a tetanus is backfired into the cord, the central positivity is increased and prolonged and the potential develops a two-part contour (Fig. 9) in keeping with the general pattern that holds for the after-potentials following tetani in all nerve fibers. The distinguishing feature in positive after-potentials is the first positive notch. In the motor neuron potential the notch corresponds to the one which is characteristic of A fibers.

That the locus of production of the central positive potential includes that part of the neuron in which the motor impulses are set up follows from the fact shown by Eccles and Pritchard that a motor reflex discharge is conditioned throughout the period during which the positive potential following a backfired volley persists. Records from a repetition of the backfiring conditioning experiment are shown in Fig. 10. They make it clearly evident that throughout the positive potential the excitability of the motor neurons is subnormal. A full-sized reflex is obtained only when the positive period is cleared.

The positive potential in the motor neurons not only has the duration of the positive after-potential in motor axons, but it is attended by the expected subnormality. There is indeed no reason for not calling it the positive after-potential of the neurons. The interpretation of the negative part of the central potential, on the other hand, is not so clear. In the experiment pictured in Fig. 10 there was nothing resembling supernormality; nor have we seen supernormality in other experiments. Recovery takes place along a continuously rising curve, just as it does in the internuncial neurons,[7] without separation of the refractory period and the subnormal period by a transient period of low thresholds. One may question whether there is any negative after-potential included in the recorded negativity. If there is, there must be an additional factor controlling excitability which has not yet been resolved. Quite possibly also under other conditions the finding may be different, as Eccles and Pritchard have described low thresholds during the negative period.

The peripheral endings of afferent fibers, which in a morphological sense are dendritic, provide another place where the conditioning effect of an antidromic volley can be tested. Here, too, there is a subnormal period. The number of impulses set up by a controlled tap on the skin is reduced as

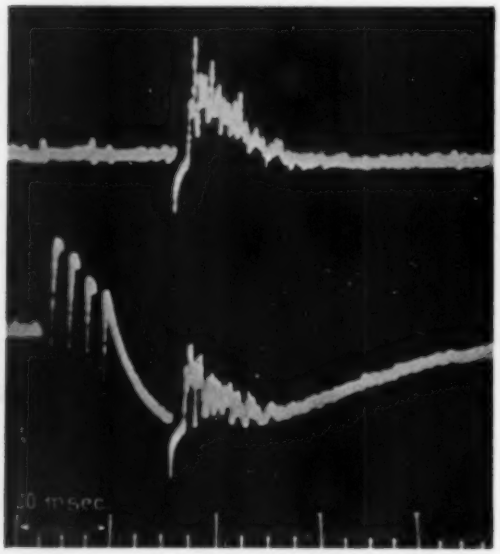

FIG. 11. Monophasic lead from the central end of a branch of the saphenous nerve of the cat. A gentle tap was applied to the skin of the leg with a device prepared by Toennies from a loud-speaker cone, and the discharge of impulses set up by the sensory endings recorded from the nerve (upper record). The discharges were reproducible as to size. In the lower record the size of the discharge has been conditioned by four volleys backfired into the periphery from a stimulating electrode on the nerve distal to the leads. The record shows four negative after potentials and the ensuing positive after-potential upon which is written the action potential of the conditioned discharge from the endings.

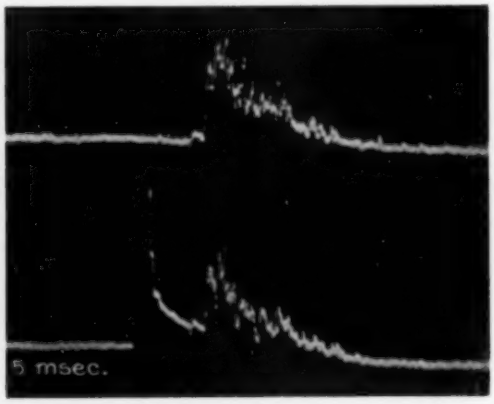

FIG. 12. Arrangement of experiment as for Fig. 11. Afferent discharge conditioned by a single volley backfired into the periphery. A discharge set up from the endings at the time when the nerve would be supernormal contains fewer than the number of impulses in the normal control.

long after the arrival of a backfired volley as the positive after-potential lasts in the nerve (Fig. 11). The probability, therefore, is strong that a positive after-potential is present in the endings. Supernormality, however, is absent, as it was seen to be in the motor neurons (Fig. 12).

Subnormal excitability following single spikes and trains of spikes is characteristic of all kinds of axons and all parts of the neuron. It is also found in all parts of the central nervous system, only it is there called inhibition. The significance of subnormality in the interpretation of inhibition makes the subnormal period, first described in nerve fibers by Graham,[5] one of the most important features of nervous activity which the axon has to present to the understanding of the synapse. The axon can also contribute to the understanding of facilitation, but the mechanism involved belongs in the group of phenomena which take place below the threshold of excitation, and facilitation, therefore, falls within the province of the next speaker.

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THE INITIATION OF IMPULSES IN AXONS

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(Received for publication May 15, 1939)

IT IS QUITE generally believed that, to quote from a current textbook (Bard, 1938, p. 6), "where there are synapses conduction of excitation takes on certain characteristics that are not found elsewhere, *e.g.*, in nerve trunks composed of axons"; and that "there is some reason for attributing the peculiarities of central conduction to the synapse." These assertions, of course, hark back to Sherrington. How fundamental are the differences between nerve fiber and synapse conduction? I propose to devote my time to a consideration of that question, rather than limit my remarks exactly to the title as announced. I shall single out for consideration a few of the asserted differences with which my laboratory has had some experience, namely, latency, one-way transmission, repetition, temporal summation or facilitation and transmission of the action potential across a nonconducting gap.

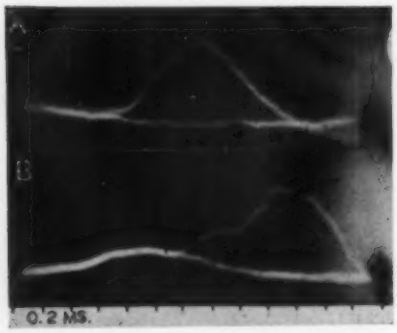

FIG. 1. Configuration changes in an axon spike at the locus of anode polarization.

A. The normal (unpolarized) diphasic axon spike on its bent base line.

B. *The higher spike* is the same axon spike, polarized anodally to the limit still permitting of conduction. The notches on the ascending limb (in this case somewhat atypical in spacings) are determined by loci of greatest susceptibility (nodes of Ranvier) of the fiber to anode polarization. *The lower spike* is the same spike after block at the first notch. The starts of the two spikes (blocked and unblocked) are superimposed. Block is indicated by the change from diphasicity to monophasicity of response. The lag at the node, due to the developing block, is of the order of 0.6 msec. *Time* is indicated in 0.2 msec. intervals.

To take up latency first, the action potential ceases to pass an anode block in a nerve fiber when the impulse is delayed there for an interval that is slightly longer than the time to maximum of the action potential. In the case reproduced as Fig. 1, for example, the block develops when the action potential lag amounts to about 0.6 msec. It seems safe to infer on the basis of observations on the relation of the duration of the action potential to conduction rate (Blair and Erlanger, 1933), that the time to maximum of the spikes in fibers of the sizes that ultimately reach the frog's neuromuscular synapse ranges between 0.3 and 3.0 msec. These figures about encompass the range of neuromuscular delays one finds in the literature. Granting block delay as the main factor determining latency, it follows that about the whole of the spike potential is needed for transmission at the neuromuscular synapse.

To pass to the next "peculiarity" of the synapse, namely, one-way trans-

mission, it has long been known (see Bishop and Erlanger, 1926) that the direction of conduction in nerve can be controlled by means of polarization. An impulse that can just pass when it progresses through a stretch of nerve successive parts of which are normal, anodally and cathodally polarized, and normal again, may be blocked when the succession is reversed, that is, from normal to cathodal, anodal and normal (Bishop and Erlanger, 1926). Bar-

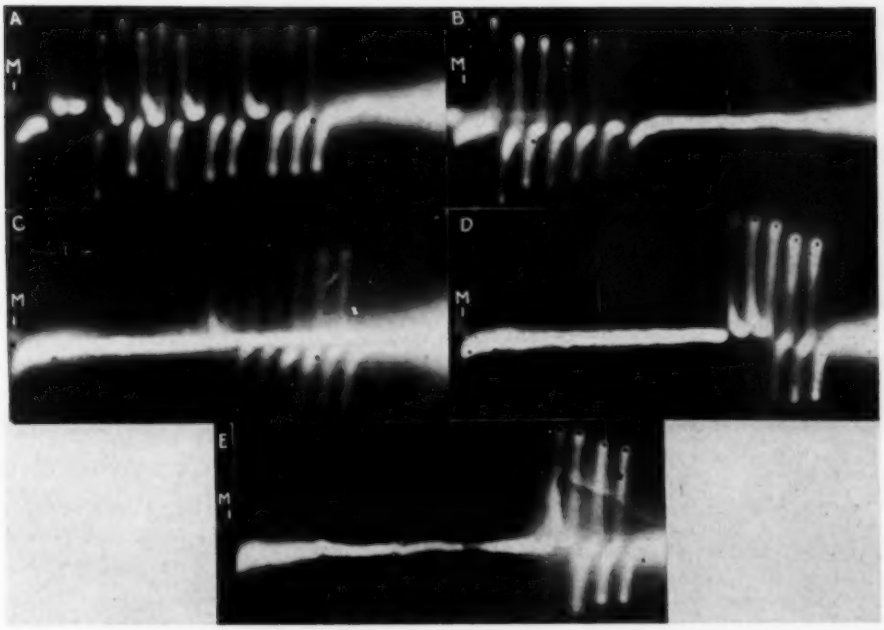

FIG. 2. The effect of continuous anode polarization on the repetitive response of a fiber elicited at the cathode of a rectangular constant current. (During this experiment the ability of the fiber to repeat was diminishing progressively; this gradual change was not the result of the increase in the strength of the anode polarization.)

A. Repetition elicited in response to the rectangular current alone.

B–E. Prior to each of the records the continuous anode polarization was increased in strength. The added rectangular current starts at M in each case. *Time* for all records is linear and may be estimated by the distance from M to the end of record in E, which subtends 72 msec. The record also demonstrates facilitation by blocked nerve impulses; fortuitously the spikes often fail to reach the distal lead at first, as evidenced by their monophasicity; but the longer each of the records runs the more frequently is the spike conducted to the distal lead.

ron and Matthews (1939) believe that in the cord the direction of conduction through the synapse may not be fixed. If it is not, changes in a spatial sequence of excitabilities, such as can be effected in fibers by polarization, might very well determine the direction of readier conduction.

As for repetition, it has been known since the time of Pflüger that a nerve may respond repetitively during polarization with a constant current. The bursts of repetition thus started in a fiber as the cathode of a rectangular current can in some measure be controlled by varying the degree of steady

anodal polarization obtaining at the time the rectangular current is started (Erlanger and Blair, 1936), as may be seen in Fig. 2. Bronk (Bronk, Larrabee and Brink, 1938) undoubtedly will refer to experiments performed in his laboratory which have shown that alteration of the Ca and K content of peripheral axons may cause them to fire off just as does a motor nerve cell. Any other method of reducing accommodation should tend to make the nerve response repetitive.

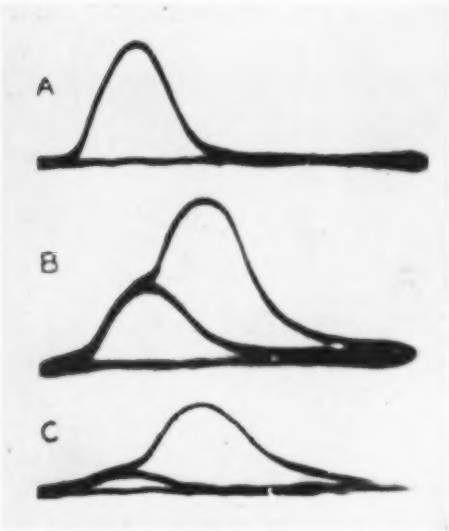

FIG. 3. Typical changes in the configuration of a monophasic axon spike produced by anode polarization at the proximal recording lead.

A. The normal spike.

B. The spike under anode polarization just strong enough to block at the most accessible node. Spontaneous variations in the fiber's excitability cause the block to fluctuate between one that is just not complete (the notched spike) and one that is just complete (the unnotched spike).

C. Further increase in the polarizing current to the next critical strength converts the unnotched spike of B into the notched spike of C, and when the latter is again blocked it records as the unnotched spike of C. The *time* is linear and may be estimated from the time to maximum of *A*, which is 0.37 msec.

We have yet to consider temporal summation and the transmission of impulses across a nonresponding gap. The rest of my time I shall devote to these topics.

To demonstrate temporal summation of subthreshold action potentials in a nerve fiber we have proceeded as follows (Blair and Erlanger, 1936). By continuous polarization of the small phalangeal nerve with the anode at the proximal lead, as indicated in Fig. 4, the impulse traveling along a particular fiber is blocked anodally at the lead. Electrical blocks develop at nodes of Ranvier, and as the polarizing current is increased, the first node to block would be the one most directly in the path of the current, and then successively the less favorably placed nodes. Figure 3 illustrates typically how the picture changes as the strength of the polarizing current is increased. The action potential first increases in height, then a notch develops on the ascending limb and at the critical polarization strength the part of the spike above the notch disappears. The action potential, if diphasic, becomes monophasic (see Fig. 1 and 4). With further increase in polarization this process repeats itself at the next node removed, and so on.

Now if, when the first stage has been attained (that is, block at the most accessible node), and while it is being maintained by steady polarization (as in B 1–3, Fig. 4), a second action potential is made to follow the first, and blocked one, within a certain time range (here the interval is 3 msec.) the second action potential may be conducted through the block, as shown

here by the restoration of height and of the second phase. The first spike, though blocked, has so conditioned the blocked node (d) that a second spike is conducted through. This facilitation is maximal when the interval between the first and the second spikes lies somewhere between 2 and 4 msec., *i.e.*, when most of the refractoriness following the first response has passed off, and declines slowly with further increase in the interval, facilitation becoming inappreciable when the interval reaches 80 to 100 msec. The spike lasts only about 1 msec.

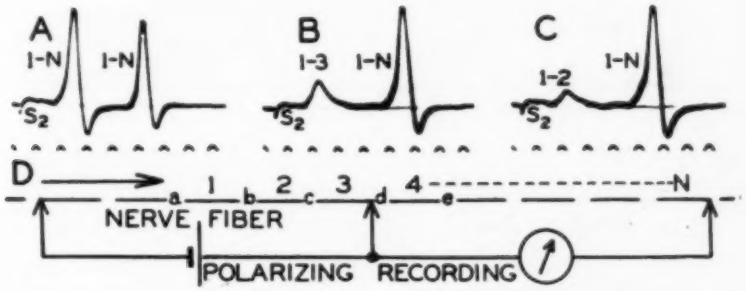

FIG. 4. Demonstration of facilitation in a nerve fiber extending the length of an internodal segment (possibly two) ahead of an impulse blocked by anode polarization. Judging by the results of the experiment, the electrode that served as anode of the "polarizing" circuit and proximal lead of the "recording" circuit occupied the indicated position relative to the nodes (a, b, c, etc.) and the internodes (1, 2, 3, etc.) of the responding fiber. With anode polarization short of blocking strength the conditioning spike recorded as 1-N in A. At the first critical blocking strength, A, 1-N changed to B, 1–3. At the second (and greater) blocking strength, B, 1–3 changed to C, 1–2. The second spike, in each case following the 1st after an interval of about 3 msec., is conducted through both blocks, at d in B, and at d and c in C.

The *time* is in milliseconds.

These values fit perfectly into the curve of temporal summation made by Bremer and Homès in 1930 through observations on the neuromuscular synapse of the frog. The preparations they used were slightly curarized, to the extent that one nerve volley failed of transmission whereas a second elicited a contraction. Their curves of contraction height against interval between nerve action potentials are reproduced here as Fig. 5. As I have said, our data derived from blocked nerve fiber fit these curves perfectly.

I have described the case where one action potential suffices to so condition the block that a second will pass. But anode blocks can be produced that are overcome by any desired number of impinging spikes, the number needed depending upon the strength of the blocking current.

Now how far beyond the anode block does the unblocking action of the blocked impulse extend along a nerve fiber? At least the length of 1 internode and in all probability the length of 2, or an estimated distance of 2 to 3 mm. An experiment showing how this has been determined is illustrated in Fig. 4. The electrode that is acting as the common proximal lead and polarizing anode has by trial been put in such a position that the normal spike, shown as 1-N in A, is converted, by a degree of polarization, into

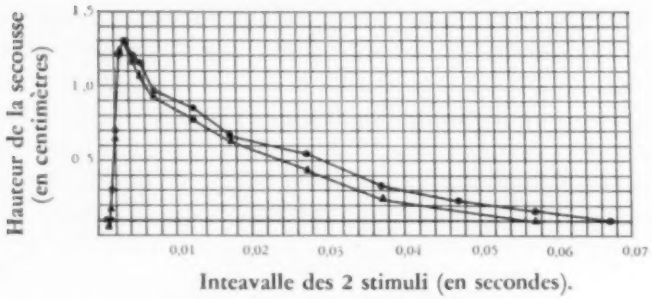

FIG. 5. Curves of temporal summation at the neuromuscular junction of the frog (from Bremer and Homès). The preparation was so curarized that the first of two successive nerve impulses failed to elicit a contraction. The height of the contraction is plotted against the interval between the two stimuli.

B(1–3) and this, by a slightly greater degree of polarization, into C(1–2). B is believed to picture anode block at node d, with restoration of conduction through d by facilitation, and C, 1–2, to picture the extension of block to node c, with restoration of conduction by facilitation not only through node d but through node c, also, giving C, 1-N. Here the conditioning influence of the action potential blocked at node c is extending from node c to node d certainly, and probably to node e.

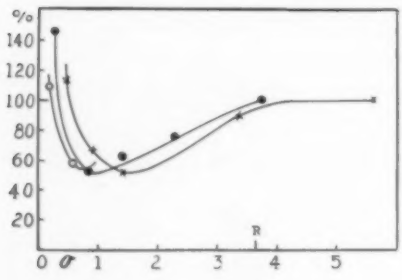

FIG. 6. Three curves showing latent addition or summation followed by postcathodal depression in in a nerve. The conditioning shock is 53, 79 and 97 per cent of threshold for the curves indicated by the circles, dots and crosses, respectively. The testing shocks, applied at the intervals shown on the abscissa, had a fixed strength such that they would under all conditions be within the submaximal range, and the height of the resulting multifiber spikes is plotted as ordinates in per cent of the spike height elicited by the testing shocks when delivered alone. The latent addition period increases from 0.2 and to 0.5 msec., approximately, as the strength of the conditioning shock increases. The subsequent 'period of postcathodal depression lasts 3–4 msec.

Hodgkin (1937) also has shown, but by another method, that the excitability of nerve is raised for a considerable distance beyond a blocked nerve impulse.

It is interesting to compare temporal summation by blocked *action potentials*, with temporal summation by two *subthreshold electrical shocks* delivered in succession through the same electrodes. Previous observations (Erlanger and Blair, 1931) on multifiber preparations have shown in response to a subthreshold shock (see Fig. 6) a summation period lasting 0.2-0.6 msec. followed usually by a depression interval, a period of postcathodal depression, lasting 3-4 msec. Blair recently (1938) has plotted for the most irritable fiber of the phalangeal preparation the local changes in threshold following the delivery of a subthreshold shock. This was done with the fiber in three states, namely, normal, anodally polarized and cathodally polarized. Sample curves are shown in Fig. 7. Under all three conditions there is, of course, a summation

period. But in this experiment only the cathodally polarized fiber exhibits the previously described period of postcathodal depression. Inconstancy of the period of postcathodal depression had been noted previously, but the factors responsible for its variability remained unknown. The state of polarization of the preparation evidently is one of them.

Another new observation, made by Blair, and also by Gasser (1938) independently, is that the period of postcathodal depression, when present, passes after 3–4 msec. into another and a long lasting, though low, summation period.

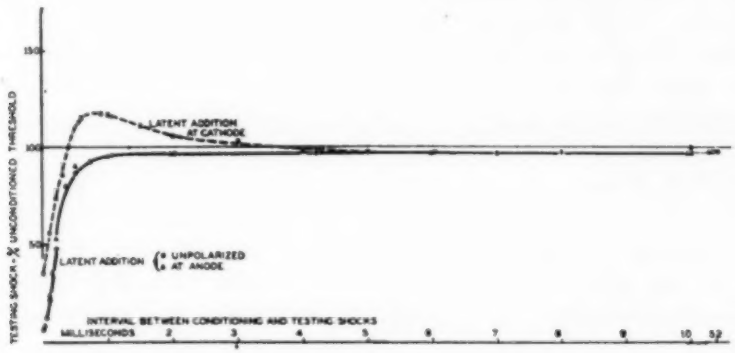

FIG. 7. Curves (exhibited and described by Blair, 1938), but not previously published) showing the effect of a subthreshold shock upon the threshold of a nerve fiber. The conditioning shocks were 94, 64 and 90 per cent of the thresholds of the fiber when normal, anodally polarized and cathodally polarized, respectively. The strength of the testing shock in per cent of the threshold of the unconditioned nerve is plotted as ordinates against the interval between the conditioning and testing shocks. The cathodally polarized fiber (squares) shows latent addition, postcathodal depression and, after 4 msec., a second period of enhanced excitability (lowered threshold) lasting beyond 50 msec. The normal (circles) and the anodally polarized (triangles) fiber does not pass through a period of postcathodal depression and remains demonstrably hyperexcitable beyond 50 msec.

In the case of the normal and of the anodally polarized fiber, as has been said, Blair found no period of postcathodal depression. Instead the latent addition or summation period is continued into a long drawn out period of slowly rising threshold. Therefore under all of the conditions that Blair dealt with, the threshold of the fiber, altered by a subthreshold shock, is below the normal level after 4 msec. have elapsed. After 50 msec. the threshold in all cases still is 1 per cent below normal.

Gasser, using multifiber, responses, has covered much of this ground independently (1938), has defined some of the conditions which will yield the long drawn out period of enhanced excitability following a subthreshold shock, has recorded associated potentials and has shown that this period of enhancement accounts for the recruitment of the fibers of a nerve when it is stimulated repetitively at rates as slow even as 20 per sec. with shocks of a constant strength initially below the threshold. Just as a considerable

number of *action potentials* may have to impinge upon a blocked locus in a nerve fiber to overcome an anode block, so, as Gasser has shown, the delivery of a number of *subthreshold shocks* through the same electrodes may be needed to cause a fiber to fire off.

Gasser's and Blair's experiments taken together then show that a subthreshold action potential would be expected to induce two periods of heightened excitability if the tissue it impinged upon were in a state resembling that produced by cathodal polarization, an immediate rise with a duration of the order of 0.4 msec., and a later rise beginning after about 3–4 msec. and lasting longer than 50 msec.; while Blair's experiments show, in addition, that if the state of the tissue resembled that induced by anodal polarization there would be but one period of summation, very intense for about 0.4 msec., but still appreciable after a lapse of more than 50 msec.

But quite as important from the standpoint of this symposium is the obvious similarity of the excitability reactions of muscle beyond a synapse to a subthreshold nerve action potential, of nerve beyond a block likewise to a subthreshold action potential, and of nerve to a succession of subthreshold electrical shocks. We may include here also Lorente's (1935) demonstration of the additive effects of induction shocks and action potentials on the excitability of motoneurons. The common denominator obviously is electrical.

Spatial summation, also, can be demonstrated in nerve fibers. Lorente, for example, has shown (1938) that subthreshold shocks may sum to produce a response when they are delivered to points as widely separated as 12–15 mm. And spatial summation of a blocked action potential and a shock has been plotted by Hodgkin (1937).

Synaptic transmission involves stimulation across a nonresponding gap and we thus far have concerned ourselves with continuous conduction, though, to be sure, with conduction made continuous through a process of facilitation. Next we wish to present evidence, now in press,[1] indicating that the nerve impulse actually can *stimulate* across a nonconducting gap in a fiber. Figure 8 is illustrative of the experiments demonstrating the phenomenon. Here the proximal lead electrode, P, is the *cathode* of a polarizing current and the two polarizing electrodes, the cathode and the anode, are separated by a distance which is slightly less than the assumed internodal distance. A separation of 1 mm. has been found to be satisfactory. This pair of electrodes is shifted on the nerve, and the current through them varied, until the desired result, to be described, is obtained. The evidence indicates that the electrodes then occupy a position with respect to two adjacent nodes (c and d) such as is depicted in the diagram. In the absence of polari-

[1] This paper has since appeared (Blair and Erlanger, 1939). In the same issue of the *American Journal of Physiology* are two papers, one by Rosenblueth and Luco (1939) and one by Luco and Rosenblueth (1939), in which results are recorded which are regarded as "incompatible with the electrical theory of neuromuscular transmission." It may be stated here that the results recorded in them in no way qualify the conclusions reached in either the present paper or in the first paper mentioned above.

zation the diphasic action potential 1 is recorded. As polarization is increased 1 changes through all gradations into 2. At a critical polarization strength the successive records then change without any regular sequence into the configurations 2 to 6. Spike 2 may change into 3 by breaking at the notch indicated by the arrow; there are no pictures intermediate in configuration between these two; the change is all-or-none. Spike 3 changes through all gradations into 5. Finally, 5 changes into 6, and this change, like that from

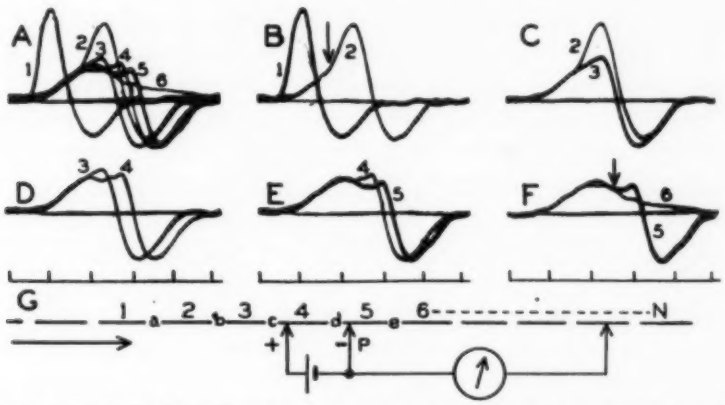

FIG. 8. Six records of the spike of an axon, selected from a large number, showing that the action potential of a fiber can be made to stimulate the fiber across the length of one, possibly of two, nonresponding internodal segments. The surmised positions of the polarizing and recording electrodes with respect to the nodes of the fiber whose spike is being recorded are shown in G. The normal spike, 1, is diphasic. At the critical polarization strength the spike's configuration, due to the fiber's spontaneously changing excitability, changes without any regular sequence as indicated by 2 to 6. There are no configurations intermediate between 2 and 3 or between 5 and 6. There is every transition between 1 and 2 as the polarization is increased to the critical level, and between 3 and 5 at the critical polarization.

C is believed to picture anode block at node c with stimulation at node d by current determined by the activity of internode 3; and F is believed to picture cathode block at node d with failure of further conduction of the impulse.

2 to 3, is without intermediate stages; it is all-or-none, the break taking place at the arrow.

The change from 2 to 3 without intermediate stages can only mean that the anode is blocking at node c. *The record* (3), *however, remains diphasic;* this must mean that despite the block the impulse continues to reach the distal lead. The potential determined by the response of internode 3 must be restimulating the fiber at node d.

Likewise, as I have said, the change from record 5 to record 6 occurs without any intermediate stages. The picture here indicates that we are dealing with cathode block; it must be at node d. This block, however, behaves as a block should; it stops the propagation of the impulse to the distal lead, witness the disappearance of diphasicity. If this interpretation of these pictures be correct, and we can see no flaw in it, we are dealing here with restimulation of the fiber at node e by potential associated with the activity

of internode 3. Two internodes, 4 and 5, have failed to fire off and yet the fiber is restimulated beyond.

Now, by way of discussion, let me recall the alternative hypotheses of neuromuscular transmission put forward by Dale, Feldberg and Vogt (1936). Either the nerve impulse directly excites the muscle cell but cannot reach its threshold unless the muscle is sensitized by a chemical substance produced *at the nerve ending*, or else a transmitter substance released *at the nerve ending* directly excites the muscle.

Transmission in a blocked nerve fiber fits neither of these alternatives. The stretch across which the axon potential can exert its effect, an internode or two, considering the time available, precludes the intervention of any process dependent upon substances released *at the nerve ending;* of any process dependent upon molecular continuity, such as secretion. The influence exerted by the potential difference upon excitability beyond the nonconducting gap alone can account for the results. It must be that if the spike itself fails to "detonate" the fiber beyond the gap, it so alters the fiber's composition there through electrochemical means that the threshold, lowered practically immediately, requires 100 msec. to return to normal. In any event, if an inactive stretch of fiber over 1 mm. in length does not stand in the way of electrical transmission of the impulse, is it reasonable to maintain that the discontinuity at a synapse will stop such transmission?

If I have succeeded in showing that there is a close correlation temporally between (i) the subthreshold effect of a shock applied to a fiber, (ii) the subthreshold effect of an action potential beyond a block at a node and (iii) the subthreshold effect of an action potential blocked at the neuromuscular junction by curari I will have succeeded in covering my assignment. If incidentally I have presented evidence indicating that an electrical mechanism can account for synaptic transmission whatever else may be involved, my defense is that it is a question of sufficient relevancy to the subject of the symposium to have justified the liberty I have taken.

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SYNAPTIC MECHANISMS IN SYMPATHETIC GANGLIA

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I

THE INTEGRATED activity of the nervous system is determined by the fixed structural relations of the constituent neurons and by the variable influence of one neuron upon another. Through the excitation of nerve cells by contiguous cells the rhythmic processes in the individual units are compounded into the pattern of activity which determines the functions of the nervous system at any moment. This intercellular relationship is established by the processes at the synaptic junctions. The nature of those processes constitutes the problem of synaptic transmission. The problem has three principal aspects.

It is at first necessary to consider the nature of the events in the presynaptic neuron, for they constitute the primary agents of excitation. Direct measurement has shown during recent years that the characteristic activity of nerve fibers is the transmission of trains of rhythmically recurring impulses (Adrian, 1932; Bronk, 1938). As these impulses reach the synapse there is accordingly a sequence of reversible changes in the terminal portions of the fiber. The character of this alteration is not invariable, for the properties of the fiber vary with the frequency of action and antecedent activity. The character and effects of an impulse in the termination of any fiber will therefore vary from time to time.

Inasmuch as a nerve impulse is a reversible alteration of the physicochemical structure of the nerve, it follows that the immediate environment of the presynaptic termination is modified by each impulse. How the properties of the synaptic region are altered by the trains of impulses which arrive at the synapse is accordingly a *second* major problem in the study of synaptic mechanisms. That can be investigated by chemical studies of the fluids which pass through this region or by measuring as an index the altered properties of the adjacent nervous tissue. By the altered environment the properties of the postsynaptic cell are modified, and under suitable conditions one or more impulses are discharged over its axon. A *third* aspect of the problem is therefore a determination of the nature of these changes in the secondary neuron which are developed by presynaptic impulses.

For the experimental study of these several phases of the problem of synaptic transmission a sympathetic ganglion is a favorable preparation. The sequence of impulses which naturally come to the ganglion from the central nervous system can readily be determined. Alternatively, the pathways from the centers may be interrupted and electrically initiated impulses sent into the ganglion over a variable number of preganglionic fibers at con-

trolled frequencies. Impulses directly excite the ganglion cells without the intervention of internuncial neurons, and their response can be measured in terms of the postganglionic impulses which they discharge. A ganglion has, furthermore, a readily isolated blood supply which enables the experimenter to perfuse with solutions of determined composition, and thus it is possible to vary the environment of the cells or to study the chemical changes produced by nerve impulses.

The results of investigations on a sympathetic ganglion cannot, of course, be applied directly to an interpretation of synaptic mechanisms in the central nervous system. There is, however, a wide-spread belief that we shall be able to learn much about the general problem from these relatively simple structures. They have accordingly been extensively employed.

In the brief space of this discussion it would not be possible to review adequately the extensive literature that has grown out of those studies. Fortunately that has been made unnecessary by the recent reviews of Brown (1937), Dale (1937), Eccles (1936, 1937, 1939), Rosenblueth (1937) and of Bronk and Brink (1939). I propose, therefore, to give a brief summary of certain results of a series of investigations which have been carried out in our laboratories during the past few years and which deal with aspects of the problem little considered by other workers. Our primary purpose has been to analyze the factors that modify the activity of a ganglion cell and which thus regulate the fluctuating patterns of nervous activity. We have given particular attention to the influence of chemical agents, to the synaptic effects of trains of impulses corresponding to those which naturally course over the presynaptic pathways, and to the prolonged modification of cellular properties developed by antecedent activity. Because of the characteristic asynchronous action of groups of neurons we have frequently found it necessary to record the events in a single unit. For the information which I have at my disposal I am indebted to my colleagues D. Y. Solandt, S. S. Tower, R. J. Pumphrey, J. B. Gaylor, Frank Brink, Jr., and especially to M. G. Larrabee whose experimental skill is responsible for much of this work.

II

The cells of a sympathetic ganglion are naturally excited by trains of impulses coming to the synapses from the central nervous system over varying numbers of fibers and at frequencies which wax and wane as the activity of the centers fluctuates under the influence of afferent stimuli (Adrian, Bronk and Phillips, 1932; Bronk, Ferguson, Margaria and Solandt, 1936). We shall see that these variations in the number of preganglionic fibers which are conducting impulses and in the frequency of their action are important factors in determining the degree of synaptic excitation.

Inasmuch as the frequency of impulses in the postganglionic neurons grades the response of the effector organ, it is important to know whether the frequency of impulses discharged from the centers is modified by transmission across the synapses of the ganglion. This is a question that has been

frequently asked (Cannon, 1914; Querido, 1924; Veach and Pereira, 1925; Bishop and Heinbecker, 1932; Knoeffel and Davis, 1933; Brown, 1934; Bronk, Tower, Solandt and Larrabee 1938). In the earlier work an answer was sought by comparing the contractions of the nictitating membrane when the preganglionic and when the postganglionic nerves of the corresponding superior cervical ganglion were stimulated alternately, at various frequencies. Although there was some disagreement among the observers as to the interpretation of the results, most of them concluded from the indirect evidence that a ganglion does not modify the frequency of incident impulses. The most direct method of answering this question, and others pertaining to synaptic transmission, would be to record the trains of impulses coming over a single preganglionic fiber to a certain ganglion cell, while recording the discharge of impulses from that cell over its postganglionic fiber. Because this is well-nigh impossible the experimental procedure has been to

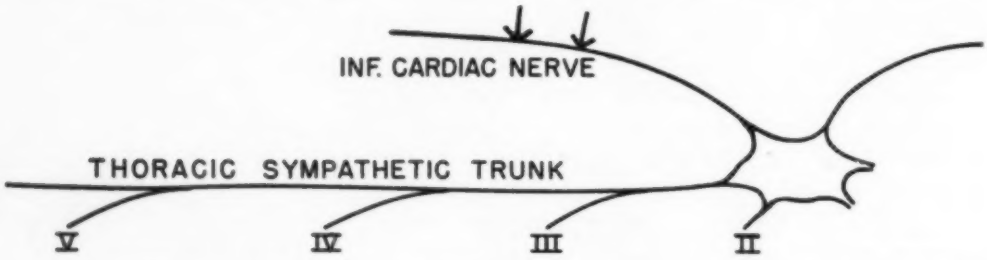

FIG. 1. Diagrammatic sketch of stellate ganglion of the cat showing the preganglionic nerves used for stimulation and the postganglionic nerve in which the cellular discharge is recorded.

stimulate the preganglionic trunk with electric shocks which thus initiate volleys of impulses in many preganglionic fibers and consequently in many postganglionic neurons.

In our own work we have usually employed the stellate ganglion of the cat,—a preparation which has many useful characteristics (Fig. 1). The first to the fifth thoracic roots as well as the preganglionic trunk are exposed and transected. It is then possible by electrically stimulating one or more of the several roots to send impulses into the ganglion over various pathways or over different numbers of fibers. The discharge from the ganglion cells, which is initiated by the preganglionic impulses, is recorded in the postganglionic fibers of the inferior cardiac nerve. The height of the spike potential recorded from many fibers is an index of the number of ganglion cells which are in action. The magnitude of the potential will, however, also vary because of changed properties of the postganglionic axons, due, for instance, to the persisting effects of previous impulses. The influence of such variables must therefore be carefully considered when the postganglionic action potential is used as a measure of variations in the activity of the ganglion cells.

The characteristic response of a group of these cells to a synchronized

preganglionic volley is a burst of impulses in the postganglionic nerve trunk (Fig. 2). The considerable width of the spike potential is due to the temporal dispersion of the individual axon spikes as they reach the recording electrodes. This dispersion is the result of differences in synaptic latencies and conduction times along the various fiber and synaptic pathways. That it is not due to a repetitive discharge of impulses from each ganglion cell is shown by records from single postganglionic fibers (Fig. 3), for these reveal a single sharp spike corresponding to a single impulse for each preganglionic stimulus (Bronk, Tower, Solandt and Larrabee, 1938). On the basis of such evidence one may say that a ganglion cell does not normally respond repetitively to a single preganglionic volley. From this we must conclude, as have Eccles (1935) and Lorente de Nó (1938), that the excitation developed by slowly-recurring volleys of presynaptic impulses does not persist at a supraliminal level after the discharge of an impulse from the cell. Synchronized volleys of impulses at frequencies not exceeding 20 per second consequently develop trains of impulses of a like frequency in each of the active postganglionic fibers.

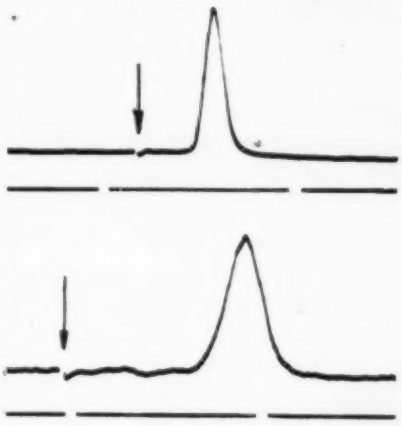

FIG. 2. Oscillographic records of the postganglionic spike potential resulting from a preganglionic volley. Recorded at 6 and 30 mm. beyond the ganglion to show increased temporal dispersion. Arrows indicate shock artefacts. Time: 0.05 sec.

Such a one-to-one correspondence between the frequency of synchronized impulses in presynaptic fibers and in postganglionic neurons does not justify the conclusion that a ganglion cell normally discharges impulses at

FIG. 3. Discharge of impulses in a single postganglionic fiber in response to preganglionic volleys. No repetitive discharge. Marker represents stimuli at 0.2 sec. intervals.

a frequency which corresponds to the frequency of action of a cell in a sympathetic center. The old and much debated question whether the rhythm of impulses from the central nervous system is modified in a ganglion cannot be answered by the use of electrically initiated volleys of synchronized impulses in many fibers. For one does not thereby initiate

the natural activity in the preganglionic nerve and the consequent sequence of events at the synapses. The use of electric stimuli for the development of groups of synchronous impulses in many fibers is a convenient and valuable means for investigating many problems relating to the excitation of nerve cells at synapses. But it must be borne in mind that the simultaneity of impulses in many fibers is unnatural.

A ganglion cell receives the terminations of many preganglionic fibers. Normally these several fibers conduct from the centers to the synapses trains of impulses, which are of different frequencies in the different fibers. The simultaneous arrival of impulses at a cell over two or more fibers is therefore only a chance occurrence. It is furthermore believed by many that the discharge of an impulse from a cell can be initiated not by a single presynaptic impulse but only by the summated effects of a number of impulses. We must therefore conclude that the frequency of cellular discharge is determined not by the frequency of impulses in any one presynaptic fiber, but by the level of "excitatory state" developed by the unrelated rhythms in a number of fibers. The suggestion that a sympathetic ganglion may modify the frequency of impulses coming from the central nervous system is undoubtedly correct.

III

There is considerable evidence that nerve cells are usually activated only by the summated effects of several impulses. These impulses may arrive at

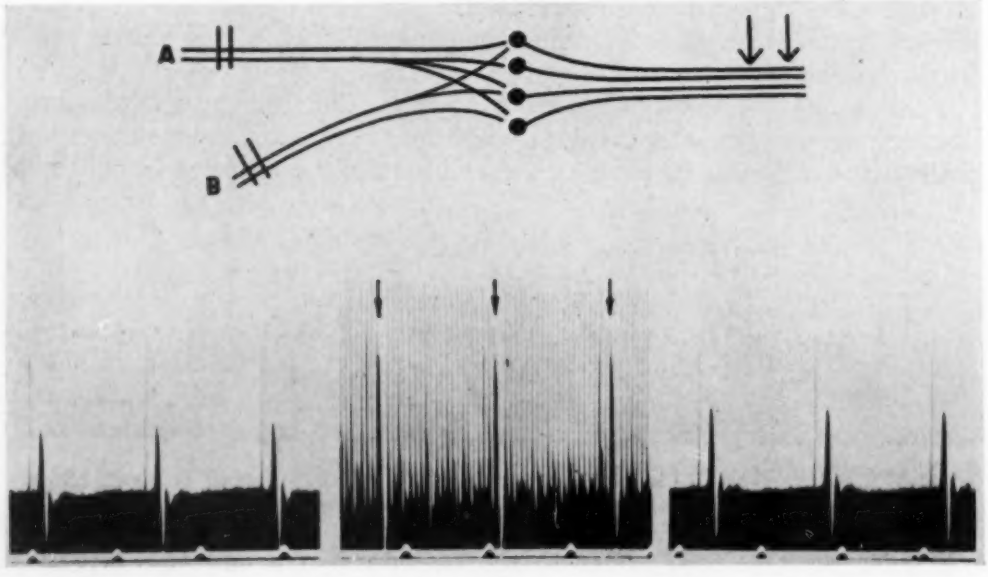

FIG. 4. Above: Schematic representation of the innervation of ganglion cells by fibers from different roots. Below: First record: Postganglionic responses to preganglionic volleys in root A. Second record: Arrows indicate responses to similar volleys during concurrent repetitive stimulation of root B. Last record: Stimulation of root A alone. Time: 0.2 sec.

the cell over different presynaptic fibers or seriatim in the same fiber. Whether a ganglion cell is brought into action may depend therefore upon the number of presynaptic fibers conducting impulses to it. This is well illustrated by Fig. 4 which shows in the first record the postganglionic discharge developed by stimulation of one root. The height of the spike potential is determined by the number of ganglion cells that are active. In the second record, similar volleys were sent into the ganglion over root A during repetitive stimulation of root B. The increased responses to the volleys in root A show that certain additional cells were activated by the summation of the effects produced by

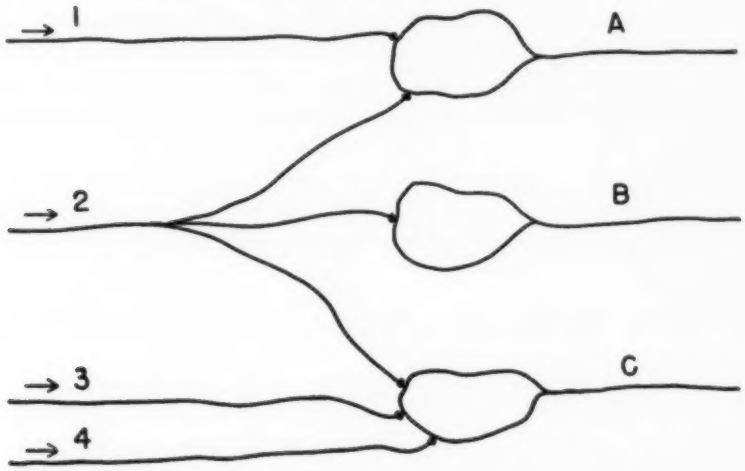

FIG. 5. Schema illustrating the control of alternative pathways in the nervous system.

the train of impulses in one set of endings with the effects developed by the volleys in another group of fibers. This example of the well-known phenomenon of spatial summation is cited here partly to emphasize the multiple innervation of ganglion cells (see also Eccles, 1935a and b). It also illustrates in a simple manner how the effect of a train of impulses on a group of nerve cells is modified by the concurrent arrival at the same group of cells of a train of impulses from another source. Whether in Fig. 5 an impulse arriving over fiber 2 will excite cells A and C may depend upon the activity in fiber 1 and in fibers 3 and 4. By such means the paths of impulses in the nervous system are undoubtedly being shifted from moment to moment with consequent alterations in the pattern of activity.

The excitation of a ganglion cell may also depend upon the frequency with which impulses arrive at one or more of the synapses. If the excitation developed by a presynaptic impulse persists for some time, the subliminal effects produced by each of a series of presynaptic volleys should ultimately summate to a threshold level. This is indeed what happens. Figure 6 shows the progressive increase in the heights of the postganglionic spike potentials initiated by a train of uniform preganglionic volleys. Inasmuch as the

magnitude of the spike potential in the postganglionic nerve is a measure of the number of fibers which are conducting impulses, it will be seen that there is a progressive recruitment of ganglion cells. How many additional units are brought into action during an extended period of repeated stimulation depends upon the number of cells that are at the beginning of the train subliminally excited by the incoming impulses. The degree of recruitment is accordingly greater when only a fraction of the preganglionic fibers innervating a given group of cells is excited or when the excitability of the ganglion cells is low. For the purposes of our discussion the significant fact is this. Trains of impulses such as those which constitute the messages of the nervous system tend to recruit more and more cells into action. Because of the decay of the excitatory state the number of additional cells which are thus activated is a function of the frequency of the presynaptic impulses. Inasmuch as the frequency of impulses is an important

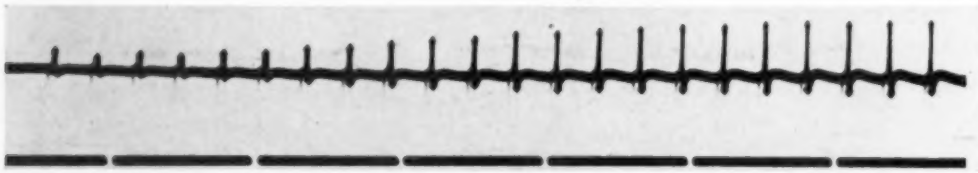

FIG. 6. Increase of postganglionic spike potentials during train of preganglionic volleys. Shows progressive recruitment of ganglion cells. Time: 0.5 sec.

variable in the messages of the nervous system, it is interesting to find that variations in frequency of presynaptic impulses modify not only the rhythm of the postsynaptic cells but also determine the number of cells in action. There will also be a selective response of certain neurons in the nervous system to certain rhythms, for the cells with higher thresholds will come into action later in a train, or respond only to higher frequencies.

IV

If the preganglionic nerve is stimulated at frequencies greater than about 20 per second, the successive volleys do not evoke progressively increasing postganglionic action potentials. On the contrary, the spikes become smaller, the rate of failure depending upon the frequency of stimulation (Bronk and Pumphrey, 1935; Rosenblueth and Simeone, 1938). This may be due in part to a progressive block of synapses by the rapidly recurring impulses. However, an examination of the discharge in individual fibers from single ganglion cells reveals another and significant reason for the decrease in height of the spike potential in the postganglionic trunk. At these high frequencies many of the cells discharge at rates which do not correspond to those of the presynaptic volleys. Because the rhythm of discharge is different in the various cells there is a temporal dispersion of the postganglionic impulses with a consequent decrease in the height of the synchronized spike potential.

We do not at the present time have much information concerning the factors that govern the frequency of this independent rhythm of a ganglion cell, but we may assume that a certain level of "excitatory state" is developed by any given number and frequency of impulses. This causes the cells to discharge at rates which are governed by the degree of excitation and by the characteristics of the individual units. The frequency of impulses necessary to produce these effects is greater than any we have observed coming from the sympathetic centers. But, the resulting discharge of impulses at frequencies not directly related to the frequency in any one presynaptic fiber and at unrelated rhythms in the several postganglionic units is similar to that of a ganglion in normal activity.

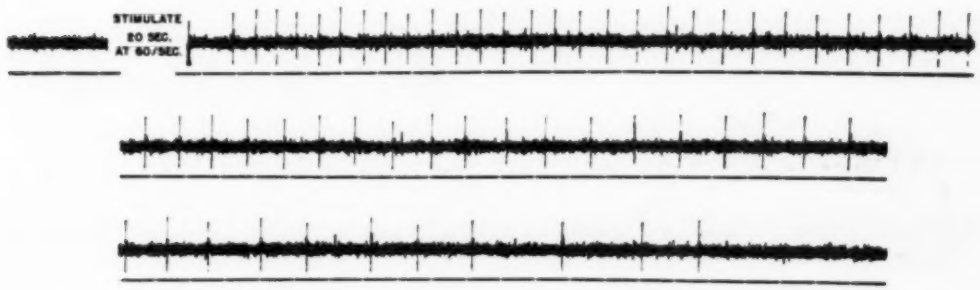

FIG. 7. Repetitive after-discharge from a ganglion cell following 20 sec. of preganglionic stimulation at 60 per sec. Continuous record. Last impulse 27 sec. after end of stimulation. Time: 0.5 sec. (Larrabee and Bronk, 1938)

Many cells which have been discharging impulses in response to a train of rapidly recurring preganglionic volleys (50 or more per sec.) continue in action for some time after the end of the preganglionic stimulation. Figure 7 is a record of such an after-discharge of impulses from a cell which continued its rhythmic activity for 27 sec. after the termination of the stimulus. Only gradually did the frequency decline, due presumably to the gradual decay of the "excitatory state." The duration of this after-discharge is graded by the frequency and duration of the previous excitation. Because it seldom appears following frequencies less than 50 per second it must be considered a clue to the nature of synaptic processes and not a phenomenon associated with a naturally activated ganglion. Whether the prolonged action is due to the persistence in the cellular environment of some agent of transmission or whether it is due to the continued liberation of some substance from the presynaptic terminations cannot be stated at the present time. The latter possibility is however reminiscent of Lorente de Nó's report (1938) of the prolonged liberation of acetylcholine following tetanic stimulation of a preganglionic nerve.

V

Even more enduring effects of synaptic excitation are revealed by the following type of experiment. A volley of impulses is sent into the ganglion,

and the height of the resulting postganglionic spike potential is recorded as a measure of the number of ganglion cells activated by the preganglionic stimulus. Similar volleys are then repeated at recurring intervals for a limited time. At various instants after the end of the repetitive stimulation the response of the ganglion cells is again tested with the same intensity of preganglionic stimulus. Such tests show that the number of ganglion cells activated by a certain number of preganglionic impulses is much increased by the intervening activity. The degree of this increase and the extent of time during which it can be observed are functions of the frequency and duration of the conditioning train of impulses. In a certain case represented in Fig. 8 the number of cells responding to the preganglionic volley was increased two-fold by a 5-second train of impulses, and only after more than

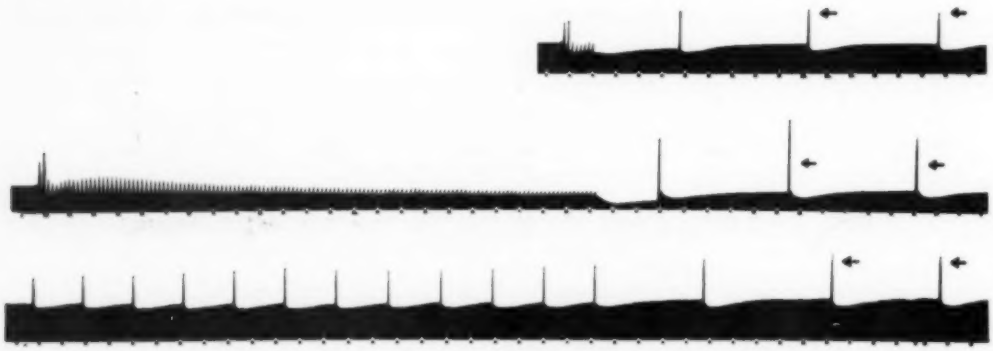

FIG. 8. Postganglionic responses to preganglionic volleys of constant size. Spike height is measure of number of cells responding. Three records showing increased response due to previous stimulation of varying frequency and duration. Time: 0.2 sec.

a minute was the postganglionic response again as small as it had been before the conditioning stimulation.

Although we assume that the conditions which make the preganglionic volleys more effective are developing progressively throughout the period of repetitive stimulation, there is during that time actually a decrease in the height of the successive postganglionic spike potentials. This need not, however, be considered as evidence against the recruitment of cells which did not respond to the initial volleys in the train. For as new cells come into action, those which have responded to the earlier volleys may drop out of action, or may respond only occasionally, because of an elevation of threshold produced by repetitive activity. The height of the postganglionic spike potential may be further reduced by the temporal dispersion described in a previous section. After the end of the repetitive stimulation, the elevation of threshold and the temporal dispersion must be assumed to disappear more rapidly than certain other changes within the ganglion. These latter changes are then revealed by an increase in the number of cells responding to a single preganglionic volley.

This prolonged increase in the response to presynaptic impulses might be due to a temporary modification of the properties of the presynaptic fibers, induced by the preceding impulses which they have conducted, There is certainly a persistent positive after-potential following activity, and with a concurrent increase in the axon spike potentials. It is possible that those characteristics of the impulse responsible for synaptic transmission would accordingly be more effective. The prolonged facilitation might, on the other hand, be due to a persistent change in the environment of the nerve cells, or to changes in the postsynaptic cells resulting from their previous activity. If antidromic stimulation modifies a ganglion cell as does stimulation through its synapses, we may rule out the last of these three factors. For

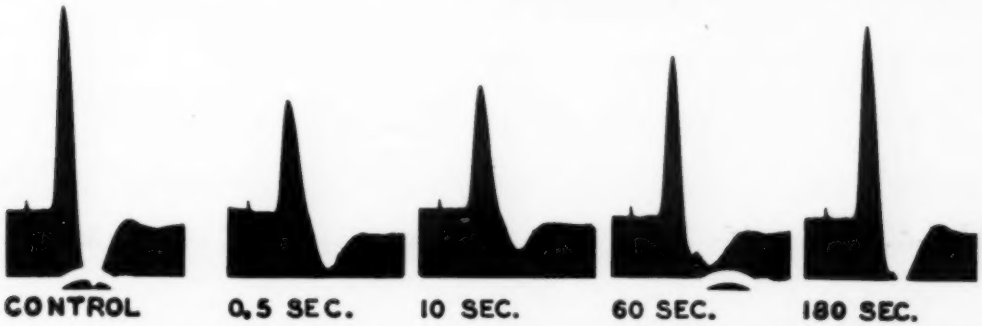

FIG. 9. Postganglionic action potentials developed by preganglionic volleys of constant magnitude. Before and at the various indicated intervals after antidromic stimulation for 1 min. at 20 per sec.

such excitation does not increase the number of cells which respond to a preganglionic stimulus.

On the contrary, the activity of a ganglion cell which is induced by stimulation of the postganglionic nerve decreases the capacity of the cell to respond to preganglionic impulses. Eccles (1937) has shown that the number of cells activated by a preganglionic volley is reduced for more than half a second after a previous antidromic volley. We confirm this and find that the postganglionic response may be decreased for some minutes following a train of antidromic impulses (Fig. 9).

Such a depression of the synaptic excitability of a cell must also occur as a result of activity initiated by presynaptic impulses. Under these circumstances, however, the reduced excitability of the cell does not cause a decreased postganglionic response, because it is masked by the increased effectiveness of preganglionic impulses. The train of impulses that initiates activity in the ganglion cells and thus causes a subsequent lowering of their excitability also develops conditions which increase the effectiveness of succeeding presynaptic impulses. If the time course of the two processes is not the same, the degree of facilitation may increase for a while following the end of the conditioning stimulus.

VI

Any analysis of the mechanisms responsible for the transient excitation produced by a single volley of presynaptic impulses, or for the more persistent modifications in the properties of a ganglion developed by a train of impulses, involves two considerations. One relates to the intrinsic changes in the preganglionic and postganglionic neurons which accompany their activity and continue for a time; the other concerns the influence on the ganglion cells of molecular and ionic alterations in their environment produced by the presynaptic impulses. In this latter category acetylcholine and potassium are of primary interest at the present time because both have been shown to be liberated in a sympathetic ganglion by preganglionic stimulation. (Feldberg and Gaddum, 1934; Vogt, 1936).

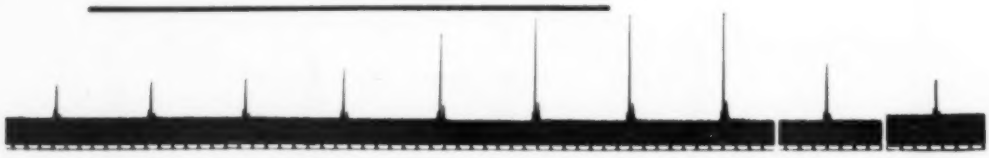

FIG. 10. Postganglionic spike potentials evoked by preganglionic volleys of constant magnitude. During upper signal 1.0 cc. of ACh was injected into perfusion fluid. Increased number of ganglion cells respond. Second record: 15 sec. later. Third record: 35 sec. later. Time: 0.2 sec. (Bronk, Tower, Solandt and Larrabee, 1938)

When a low concentration of acetylcholine in Ringer's fluid (Feldberg and Gaddum, 1934) circulates through a ganglion the number of cells responding to a volley of presynaptic impulses is increased. This is shown in Fig. 10, and the same effect is produced by increasing the concentration of potassium ions in the perfusion fluid. Whether these agents augment the response by increasing the excitability of the ganglion cells, or whether they modify some other factors in the synaptic mechanism, we do not know. In any event, their action is such as could explain the long persisting facilitation following a train of presynaptic impulses, provided it is shown that the acetylcholine and potassium outlast the period of excitation or continue to be liberated. We may also conclude that the liberation of acetylcholine by presynaptic impulses would have at least an important adjuvant action in synaptic transmission.

That there is a direct stimulating effect of acetylcholine on nerve cells is well known, for when it is perfused through the superior cervical ganglion the corresponding nictitating membrane contracts. (Feldberg and Vartiainen, 1935). This stimulating effect has the characteristics of a localized or specific action, for the ganglion cells respond to concentrations which do not excite preganglionic fibers or fibers running through the ganglion without synapse. This is well illustrated by an experiment represented in Fig. 11, wherein the activity of both the preganglionic and postganglionic nerves was recorded. Acetylcholine in a concentration of 100 μg. per cc. of perfusion fluid caused

a vigorous discharge of impulses from the ganglion cells, whereas even five times this concentration developed no impulses in the preganglionic fibers. That the presynaptic neurons were, however, accessible to exciting agents in the perfusion fluid is shown by their response to sodium citrate.

Because a chemical agent develops unrelated activity in the different neurons of a ganglion, its action is best studied by observing the discharge of impulses from a single cell. When this is done we find that acetylcholine initiates rhythmically recurring impulses which continue as long as the level of acetylcholine is maintained by the perfusion. There is no evidence

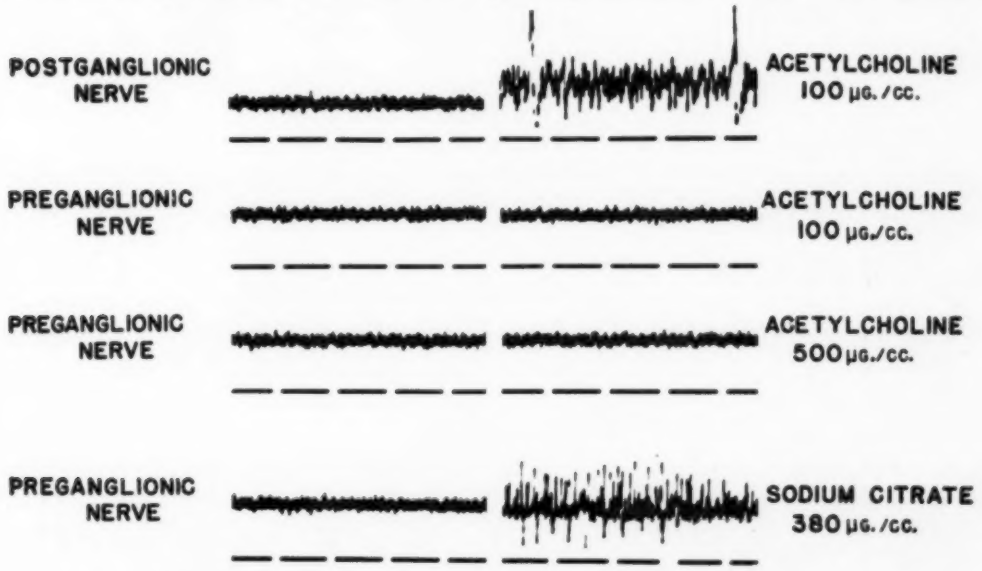

FIG. 11. Preganglionic and postganglionic responses to ACh and sodium citrate. Controls with Ringer's fluid in left hand column. Time: 0.1 sec.

of adaptation or failure for many minutes provided the concentration is below that which causes paralysis. The degree of this activity is graded by the concentration of the acetylcholine. Changes in the concentration modulate the frequency of discharge. This is shown in Fig. 12 which is the record of impulses from a cell the rhythm of which was increased from about 1 per sec. to 3 per sec. by raising the concentration of acetylcholine from 25 to 100 µg. per cc.

The effectiveness of acetylcholine and other stimulating agents varies, of course, from cell to cell. There is accordingly a wide range in the concentrations necessary to excite and in the frequency with which the cells discharge under the influence of the same concentration. Nor is the response of any one cell the same from moment to moment except under carefully controlled conditions. Variations in other chemical components of the environment and additional activity of the cell are two important factors that modify its rhythm.

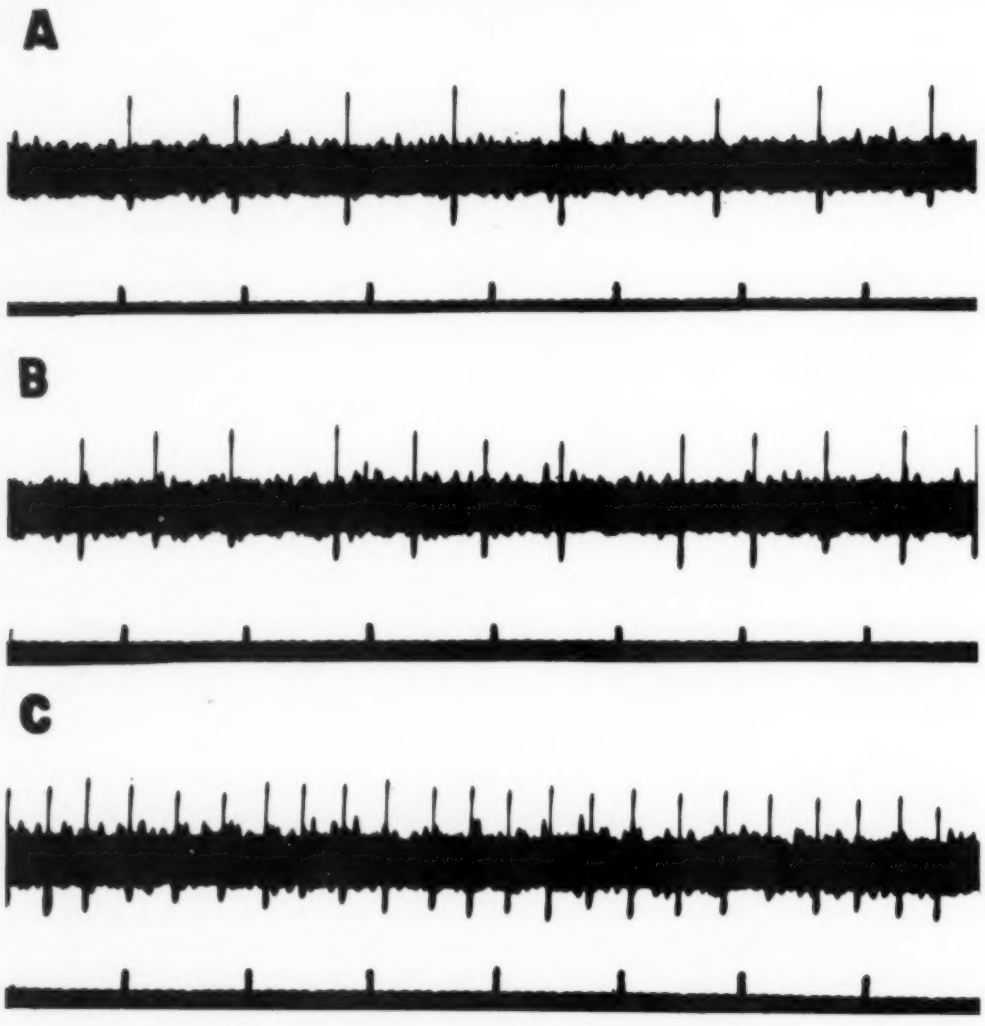

FIG. 12. Discharge from a cell in a ganglion perfused with A: 25μg.; B: 50μg. C: 100μg of ACh per cc. of Ringer's fluid. Time: 1 sec.

The activity of a cell excited by acetylcholine is for example modified by changes in the concentration of calcium or potassium in the environment. Such an effect is illustrated in Fig. 13. The second record in either column shows the impulses coming from a nerve cell in a ganglion perfused with normal Ringer's fluid containing 40 μg. of acetylcholine per cc. When the calcium concentration in the fluid was doubled the frequency of impulses discharged by the acetylcholine-activated cell was greatly reduced for several minutes. A similar reduction in the rhythm was produced by the alternative procedure of lowering the potassium. On the other hand, an increase in the concentration of potassium ions or a reduction of calcium augmented the rate of activity developed by a given amount of acetylcholine. These ex-

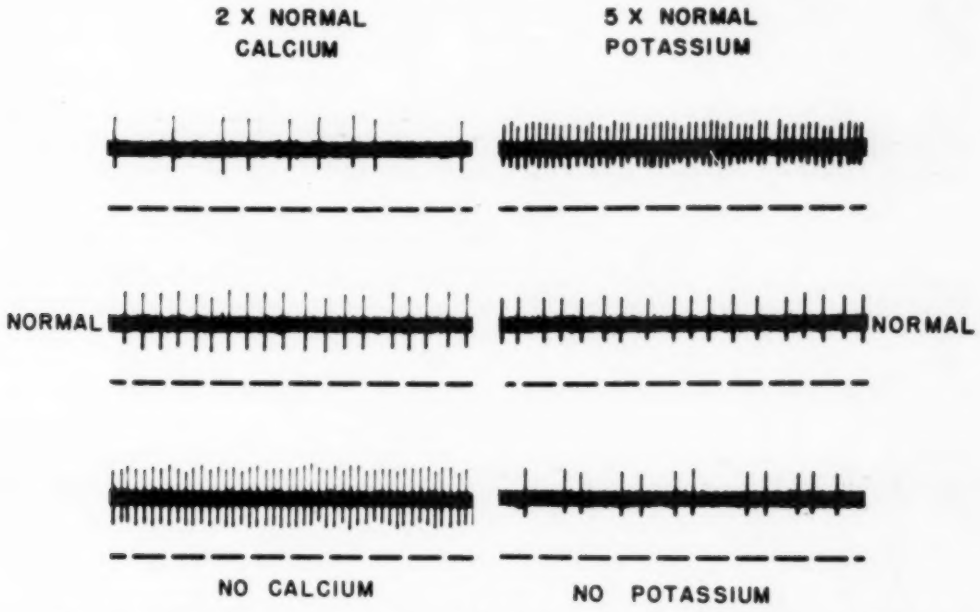

FIG. 13. Discharge of impulses from a single cell in a ganglion perfused with solutions containing 40μg of acetylcholine per cc. Middle records are with ACh in normal Ringer's fluid; others modified as indicated.

periments emphasize the general principle that the degree of activity of a nerve cell is determined by the combined effects of the various agents in the cell's environment.

VII

Another element of importance is oxygen. If the normal circulation of a ganglion is stopped or if the oxygen is removed from the perfusion fluid, the threshold of the cells for stimulation by acetylcholine or potassium is increased after a short time. Usually within the course of about ten minutes this becomes manifest as a decrease in the postganglionic discharge, due presumably to a reduction in the number of active cells and a decline in the rhythm of those which are still in action. After 30 to 60 minutes all response to the chemical agent has disappeared. No cells can then be activated even by high concentrations of potassium or acetylcholine.

During the time that the cells are losing their ability to respond to chemical excitants there is a progressive block of synaptic transmission. A gradual decrease in the height of the postganglionic spike potentials, initiated by preganglionic volleys of constant size, shows that fewer cells are being excited by the incoming impulses. Finally, the response to chemical stimuli and to presynaptic impulses is abolished at about the same time. The relative effects of asphyxia on the processes within the presynaptic terminations and on the postsynaptic cells are not known, but the parallel time course of

failure for chemical and synaptic excitation suggests that the irritability of the cell fails as soon as any part of the synaptic mechanism.

Schröeder (1907), Cannon and Burkett (1913) Bronk and Larrabee (1937) and Bargeton (1938) have pointed out that the failure of ganglionic transmission is partially reversible after somewhat more than an hour of complete anemia. Such a revival of excitation at a synapse is shown in Fig. 14. The arrest of circulation through a perfused ganglion had in that experiment produced the characteristic and progressive block in an increasing number of synaptic pathways. Transmission was finally abolished in all units after about 60 minutes and remained absent during the succeeding 6.5 hr.

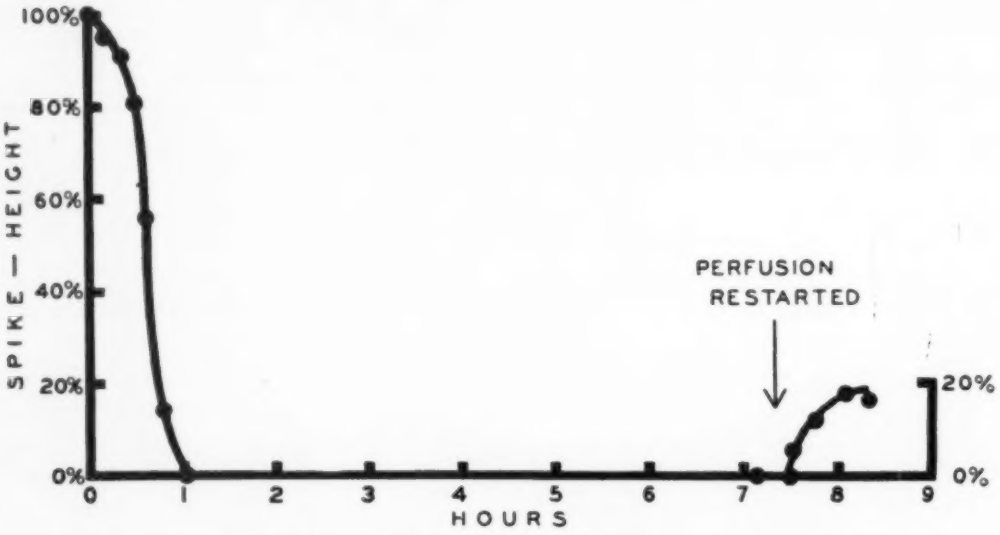

FIG. 14. Failure of ganglionic transmission during arrested circulation; followed by partial recovery.

Perfusion was then started again and within a brief interval 20 per cent of the cells were again responding to the preganglionic volleys. These experiments show that the properties of the presynaptic and postsynaptic units involved in the process of junctional transmission can be regained after being inoperative for more than six hours under asphyxia.

It has been widely held that transmission across synapses is readily blocked by asphyxia. We were therefore surprised to find that conduction over those preganglionic fibers which course uninterruptedly through the ganglion fails as soon as does transmission over the pathways containing synapses. The fact was established in the following manner (Larrabee, Gaylor and Bronk, 1939). At one minute intervals maximal shocks were applied to the preganglionic trunk while recording the resulting volleys in the inferior cardiac or cervical sympathetic nerves. From the latter nerve especially two distinct groups of impulses are recorded; the first comprises impulses in fibers which can be shown to pass continuously through the

ganglion, the later is the discharge from cells within the ganglion. The time course of failure for both of these is about the same. From this we infer that the processes involved in synaptic transmission are no more susceptible to lack of oxygen than are those responsible for conduction over certain axons.

The persistence of synaptic transmission in sympathetic ganglia throughout many minutes of asphyxia appears to be in marked contrast with the rapid failure of activity in the central nervous system. This is probably largely due to differences in the rate of metabolism of the nerve cells in the two locations (cf. Gerard, 1937). We must also bear in mind that the slow rate of failure of ganglionic transmission that has just been described pertains to synaptic pathways which are conducting at infrequent intervals. When they are caused to transmit trains of impulses at a frequency of five or ten a second their ability to conduct fails much sooner. Any comparison of the rate of failure of activity in the central nervous system and in sympathetic ganglia should undoubtedly be made relative to ganglia in continual activity. For that is the normal condition of many cells in the centers and in the ganglia as well.

VIII

This is another instance of the necessity for considering the effects of repetitive activity on synaptic processes when one is analyzing the mechanisms of this junctional transmission. The activity of a postsynaptic neuron is regulated not only by the rhythmic impulses in the presynaptic terminations, but also by the previous activity of the postsynaptic cell itself. Antecedent activity is accordingly an important factor in the determination of the fluctuating patterns of nervous action within an anatomically fixed system of neurons. The ultimate effects of a train of impulses in a neuron depend upon the irritability of the nerve cells which it innervates, and that is profoundly modified by previous events.

In a series of beautiful experiments Gasser and his colleagues (1937a, 1937b) have traced the time course of irritability changes in peripheral nerve following the conduction of a train of impulses, and have correlated the irritability cycle with the sequence of after-potentials. They thus find that an increased negativity of a region of a nerve is an index of increased irritability, whereas the irritability is decreased during the positive phase. The after-potentials are accordingly a valuable measure of the excitability of nerve.

By the use of various electrical leads from the ganglion or from the ganglion and the postganglionic nerve Eccles (1935a, b, and c) has similarly attempted to follow the altered properties of the ganglion cells which are induced by a previous impulse. Whether the variations in potential difference thus recorded actually relate to the surface of the cell body or whether they are a measure of the properties of the postsynaptic cell axons within the ganglion is still a debated question. But it is not improbable that the time course of the after-potentials in both portions of the neuron is the same. In

any event, Eccles finds that the ganglion cells are more readily excited by a preganglionic volley during the negative phase of the potential cycle following a previous volley, and fewer of the cells are excited during the positive phase. This correlation of the excitability of ganglion cells to presynaptic impulses with the after-potential cycle is then analogous to the correlation of after-potential cycles in peripheral axons with their electrical excitability. This analogy might indeed be taken by some as evidence favoring synaptic transmission by circulating currents.

Rosenblueth and Simeone (1938) point out that the decreased response of the postsynaptic cells following a previous volley of impulses may be due not to a decreased excitability of the cells but to a decrease in the efficacy of

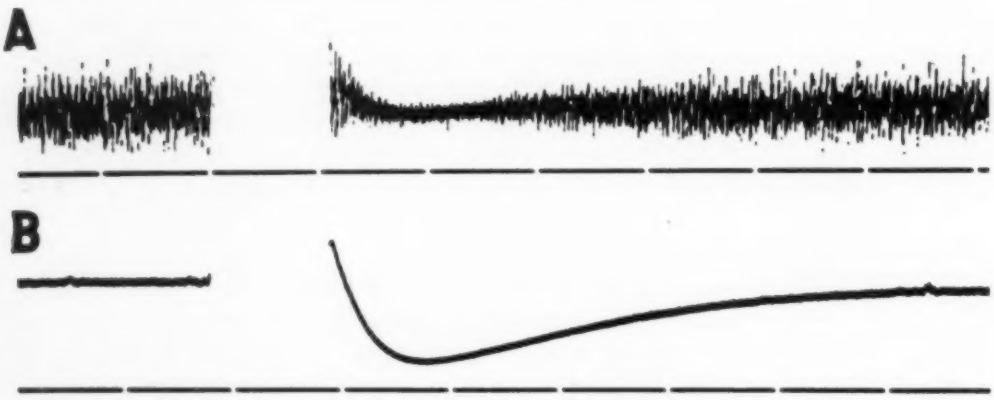

FIG. 15. Above: Discharge of impulses in postganglionic nerve during perfusion of ganglion with Ringer's fluid containing 100μg. ACh per cc. During break in record preganglionic volleys were delivered at rate of 40 per sec. Below: Ganglion potential (leads on ganglion and postganglionic nerve). Time: 0.5 sec.

the preganglionic test volley. They thus emphasize an important factor to which I have often referred in the present communication. It is certainly necessary to recognize as they do that activity in presynaptic fibers modifies their subsequent properties, and may therefore alter the effects produced at the synapse by succeeding preganglionic impulses. Because of such considerations and because in their own work they find a lack of correlation between the after-potentials and the responsiveness of the ganglion cells, Rosenblueth and Simeone question whether there is a general relation between ganglion potentials and synaptic excitability.

They furthermore raise the pertinent issue: "if nerve impulses do not act at synapses as electrical stimuli but excite the succeeding neurons by some other mechanism, then it need not be expected that the two after-potential phases should be attended by increased or decreased responsiveness. . . . For it is not known whether or not the threshold of the ganglion cells to acetylcholine varies during the after-potentials, or during the post-tetanic period. Such knowledge may provide a test for the (chemical) theory." This is an

important question which leads us back to a consideration of factors which modify the chemical excitability of ganglion cells.

We have already described how the response of the cells to a chemical agent such as acetylcholine is altered by the presence of other substances. The persistent influence of activity is now shown in Fig. 15A. At the left is the beginning of a record of the impulses discharged from many cells in a ganglion perfused with Ringer's fluid containing 100 μg. of acetylcholine per cc. We then sent volleys of impulses through the ganglion at the rate of 40 per sec. for half a second. This is shown as a break in the record, for the magnitude of the synchronized postganglionic spike potentials was sufficient to carry the electron beam off the face of the oscillograph. Immediately after the end of the train of impulse volleys there was a brief increase in the response of the ganglion cells to acetylcholine, and then an almost complete cessation of the discharge. Not for three seconds did the activity increase to what it had been before the cells were excited by the train of preganglionic impulses, Such experiments show that additional activity alters for some time the response of nerve cells to chemical agents.

Whether this sequence of changes in chemical excitability of the ganglion cells is related to a cycle of ganglionic after-potentials is answered by a comparison of Fig. 15A and B. The latter is a record of the ganglion potential which shows a brief initial negative phase followed by an increased positivity which lasts for some seconds. It is readily apparent that the increased discharge of impulses from the cells which has been described comes during the period when the ganglion is negative, relative to inactive tissue: the decreased activity during the period of positivity. There is accordingly a remarkable parallelism between the cycle of ganglionic after-potentials and the chemical excitability of ganglion cells. Inasmuch as the correlation between the sequence of after-potentials and the electrical irritability cycle of axons corresponds to that between ganglion potentials and the chemical excitability of ganglion cells, we can probably hope for no test here that will decide between the electrical and chemical theories of synaptic transmission.

It has been shown by Grundfest and Gasser (1938) and by workers in our laboratory that the magnitude and duration of the after-poentials of axons are determined by the frequency and duration of the preceding trains of impulses. The corresponding irritability changes are similarly regulated. Inasmuch as Rosenblueth and Simeone (1938) observe that the ganglionic after-potentials are likewise varied by the frequency and duration of the conditioning stimulus, we might expect to find that the frequency of discharge from chemically excited ganglion cells can be graded in a corresponding manner.

For such an analysis it is necessary to follow the activity of a single neuron. This has been done in experiments such as that represented in Fig. 16. In both A and B the upper record is of the ganglion potentials. The pronounced after-positivity is seen following the train of impulses sent in over the preganglionic trunk. How the magnitude of that potential can be graded

by the frequency of the conditioning volleys is strikingly illustrated by a comparison of A in which the stimulus was at the rate of 9 per sec. and B in which the rate was four times as great. The changed properties of the ganglion cells, which are revealed by these ganglion potentials, modify the cellular response to a chemical excitant such as acetylcholine. This is evident from the lower records of Fig. 16 A and B. A is the record of what appears to be the discharge from not more than two cells under the influence of a perfusion fluid containing 50 μg. of acetylcholine per cc. The activity ceases for somewhat less than two seconds during the peak of the positive after-

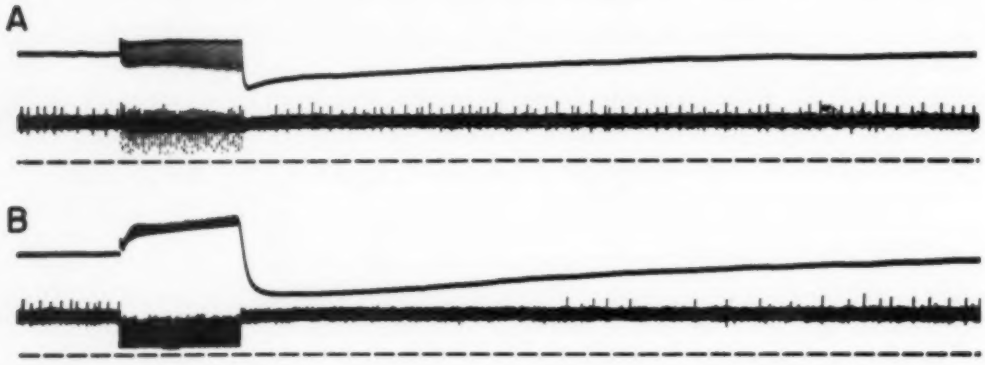

FIG. 16. Upper records in both A and B: Ganglion potentials developed by 5 sec. tetanus at rates of 9 per sec. in A and 36 per sec. in B. Lower records in both A and B: Discharge of impulses from one or two cells in the ganglion perfused with Ringer's fluid containing 50μg. ACh per cc. Inhibition of discharge during peak of positive after potential. Time: 1 sec.

potential. In B, on the other hand, the cell fails to respond to the acetylcholine for 14 seconds after the period of rapid activity induced by the preganglionic volleys. And then only gradually does the rate of chemically induced discharge return to its original level, paralleling a decrease of the positive after-potential. Such a gradation of the frequency of response of a nerve cell to a specific chemical agent by variations in the frequency of impulses coming to the cell may be of great significance in regulating nervous action. For impulse frequency is a principal variable in nerve messages, as I have said before.

These changes in the chemical excitability of a ganglion cell must be ascribed to the previous activity of the postsynaptic neuron for the effects are the same whether induced by preganglionic or by antidromic stimulation. The lowered irritability is accordingly similar to the decreased response of ganglion cells to presynaptic impulses that follows a period of postganglionic activity. It will be recalled that in the latter case the lowered excitability of the cells can be observed only after the cells have been excited antidromically. For if they are conditioned by preganglionic impulses, the presynaptic terminations or the properties of the synaptic region are altered

in such a way that the effectiveness of the subsequent test volley is increased, and the lowered excitability of the ganglion cells is thereby masked.

We must now conclude that following the propagation of one or more impulses a ganglion cell is for some time less readily excited by chemical agents including acetylcholine; by presynaptic impulses; or—to draw an analogy from peripheral nerve—by electric currents. This raises the question of the relationship between these several modes of excitation, and that brings us to a consideration of the means by which preganglionic impulses excite adjoining ganglion cells.

I have no desire to defend either the acetylcholine hypothesis or the theory of excitation by circulating currents from the presynaptic terminations. On the other hand I do not wish to oppose them or to adopt a dualistic hypothesis. If it be necessary to do more at this time than describe the phenomena of transmission and relate them into a consistent scheme, I would argue for a pluralistic theory.

If acetylcholine is liberated by impulses at the terminations of preganglionic fibers, as abundant evidence shows, there is no doubt but that the properties of the ganglion cells will thereby be altered. In sufficient concentrations acetylcholine causes the ganglion cells to discharge impulses. In weaker concentrations it increases the ability of the cells to respond to presynaptic impulses. But similar effects are produced by potassium, and it is not improbable that an incident impulse at the synapse modifies the concentration of this and other ions at the surface of the adjoining cell. And almost certainly the altered properties of the fiber terminations accompanying an impulse will give rise to a flow of current which will have some effect on the secondary neuron. Without attempting to evaluate the relative importance of any agent I would therefore urge the point of view that a presynaptic impulse modifies in many ways the environment of the contiguous cell body. The degree of excitation must then be determined by the summated effects of the various environmental factors—of those which tend to stimulate and of those which serve to depress.

It is furthermore necessary not to think of synaptic transmission as the development of one impulse by another. It is more probable, on the contrary, that a sequence of impulses in a number of fibers produces changes in the surroundings of a cell. The properties of the cell are thereby altered, and at a certain stage in the process an impulse is discharged.

Finally, the active agents in the synaptic mechanism should be considered as having variable characteristics. The presynaptic fibers conduct to the site of action, not isolated impulses, but trains of rhythmically recurring waves of activity of fluctuating frequency. Thus the properties of the fiber terminations and the characteristics of their impulses are continually changing, So, too, are the characteristics of the postsynaptic cells modified from moment to moment in accordance with their changing environment and their previous activity. By thus regulating the properties of the synaptic

junctions between nerve cells the rhythmic trains of impulses in the individual neurons create the changing patterns of nervous action.

I am greatly indebted to Doctor Martin G. Larrabee for much assistance in the preparation of this paper. The experimental work reported here for the first time has been generously supported by grants from the Supreme Council, Scottish Rite Masons and from the American Philosophical Society.

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TRANSMISSION OF IMPULSES THROUGH CRANIAL MOTOR NUCLEI*

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I. INTRODUCTION: PRIMARY CONSIDERATIONS

Differences between sympathetic ganglia and central nervous system

THE PRECEDING report of this Symposium written by Dr. Bronk deals with the transmission of impulses through sympathetic ganglia, *i.e.*, through synaptic relays of relatively simple anatomy. The present report is concerned with the transmission of impulses through organs of almost indescribable anatomical complexity,—the cranial motor nuclei and the pools of interneurons† that are connected with them. It is true that in many respects the neurons of the central nervous system are comparable to the sympathetic ganglion cells, and it is also true that the preganglionic fibers, after their entrance into the ganglion, divide and form arborizations comparable to those formed by the afferent fibers of any pool of central neurons; but the similarity between the ganglia and the central nervous system does not go far beyond these points. Among others, the following difference is fundamental. All the fibers articulated with sympathetic neurons belong to a single tract (the preganglionic trunk) and consequently may be activated simultaneously by a suitable single stimulus, for example, an electric shock; but the fibers articulated with motoneurons or interneurons as a rule belong to many different tracts and cannot be made to conduct impulses simultaneously in response to one electric shock or any other single stimulus. Moreover, the internuncial pools are reciprocally connected by fiber paths, with the twofold result: (i) that each interneuron constitutes a link in at least one chain of several neurons, and (ii) that each chain of interneurons is linked with many others.

Synaptic transmission does not necessarily follow synaptic stimulation

The significance of these anatomical facts cannot be properly understood without considering a physiological factor. The transmission of impulses through any neuron is not an event that necessarily occurs after the activation of any synapse on the neuron. On the contrary, it is an event that fails to take place unless several synapses are activated, and, moreover, unless the activation complies with a set of most rigid and exacting conditions. Figuratively speaking, it may be said that synaptic transmission is "optional" for

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† Following the example of Prof. Gerard the simple term "interneuron" will henceforth be used instead of the rather cumbersome designation "internuncial neuron."

any neuron; therefore, without detailed knowledge of the anatomical and physiological conditions that determine the "choice," it cannot be predicted whether stimulation of fibers of a certain nerve, or of a certain pathway, will

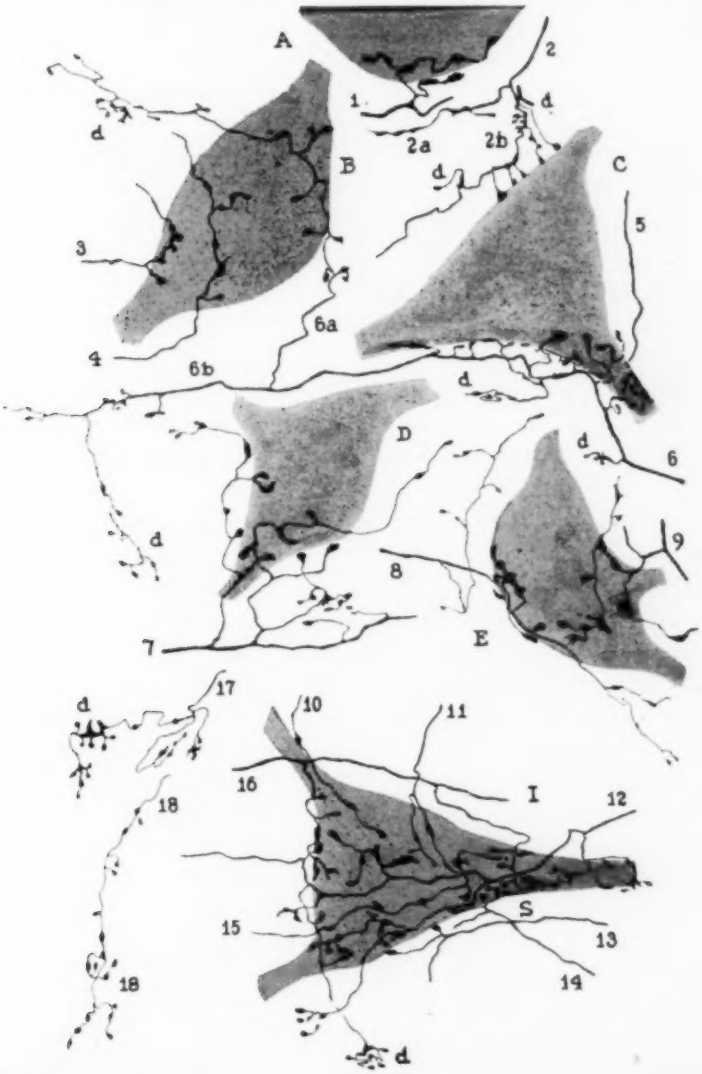

FIG. 1. Synapses on motoneurons (A to E) and on a large interneuron (I) of the spinal cord of a 15–16 day cat; *1* to *18* presynaptic fibrils. *d*, synaptic knobs in contact with dendrites. Silver-chromate method of Golgi (From Lorente de Nó, 1938c, Fig. 3).

result in transmission; nor can it be predicted through which anatomical channels the transmission, if at all, will be effected.

Study of Fig. 1 and 2 is necessary for a rigorous statement of the problem. Figure 1 illustrates the constitution of the synaptic scale on motoneurons and interneurons. *A*, *B*, *C*, *D*, and *E* are bodies of motoneurons; *I* is the body of

a similar large neuron which presumably belonged to the internuncial system. Numerals *1* to *16* indicate fibers forming synapses on those bodies and also synapses, *d*, on dendrites; fibers *17* and *18* form synaptic knobs only on dendrites. It will be noted: (i) that none of the fibers *1* to *16* has on any neuron more than a small number of knobs, which cover only a small part of the surface of the soma, and (ii) that the knobs formed by any one fiber are not close together, but separated by large spaces which are filled by knobs belonging to other fibers. For these reasons convergence on the same neuron of a number of presynaptic fibers results in the formation of a mosaic of knobs, which, except for narrow interstices between knobs, forms a continuous scale around the underlying soma. The interesting fact now is that although total activation of the synaptic scale of any neuron would demand that an enormous number of fibers conduct impulses, total activation of discrete zones of the scale may be effected by a rather small group of fibers. For example, in the case of cell *I* in Fig. 1, the stain of the synaptic scale is practically complete at the zone labelled *s*, so that total activation of this zone did result whenever fibers *12*, *14*, *15* and *16* conducted impulses. This anatomical fact, added to several experimental facts (cf. Lorente de Nó 1935*f*, 1938*c*), forms the basis for a theoretical argument.

Relevant experimental facts are: (i) Threshold stimulation of a neuron does not demand activation of all the knobs of its synaptic scale, because the stimulation by single volleys can have gradations of intensity (Fig. 5), and also because motoneurons may be caused to discharge by volleys of different constitution, *i.e.*, by impulses carried by different groups of fibers. (ii) While threshold stimulation demands convergence on the neuron of several impulses, the number of impulses is not the only determining factor. Large volleys, indeed very large ones, may remain ineffective, while a few of the impulses, which they contain, when added to impulses carried by other fibers, do set up a response. (iii) The soma of the motoneuron is electrically excitable and the nerve impulse when entering it is accompanied by an electrical sign (cf. below Fig. 14, *3*). Therefore, decremental propagation of the effects of subliminal stimulation must be expected to occur in the soma of the motoneuron. The rate of decrement is unknown but since fact ii has been demonstrated, the decrement must be high. In view of fact ii the conclusion should be that, in the case of ordinary multipolar neurons, effective summation does not take place with impulses delivered to knobs located at distances from each other greater than the distances between the knobs from any fiber, nor with a number of knobs equal to the maximal of the largest cluster formed by any one fiber (cf. the knobs of fiber *6* on cells *B* and *C* in Fig. 1). (iv) The synaptic delay cannot be reduced below a minimal value of about 0.5 msec.; this minimal interval is observed even when the effective impulses are delivered to motoneurons for which the transmission has been facilitated by the arrival of other impulses. Undoubtedly then, an impulse that has arrived at a certain synapse cannot bring to completion the excitatory process initiated at other synapses. It must start a process of its own.

In other words, impulses arriving at distant knobs may lower the threshold at a certain point, but the new impulse is not initiated unless the knob over that particular point creates its own excitatory process. (v) The effect on the neuron of impulses delivered at synapses not only must have a great spatial but also a great temporal decrement, because the effectiveness of summation of impulses arriving at different synapses, as well as the effectiveness of summation of a volley of synaptic impulses and an induction shock (Lorente de Nó, 1935*f*), declines rapidly, as the stimuli to be summated (the two volleys of impulses or the volley of impulses and the electric shock) are delivered at progressively increasing intervals of time.

Hypothesis concerning the conditions required by synaptic transmission. It seems that there is only one assumption that would satisfactorily account for these facts, namely, that threshold stimulation of the neuron takes place whenever all, or at least the majority, of knobs at a discrete zone of the neuron, as for example zone *s* in Fig. 1 *I*, are activated simultaneously, or within a very short interval of time. In agreement with this assumption it may be held, as the writer does, that the impulse is locally initiated, *i.e.* underneath the most densely activated zone of the synaptic scale, and that from there on it spreads over the remainder of the soma and also enters the axon; but other views, concerning the initiation and the spread of the new impulse would also be compatible with the basic assumption of threshold stimulation by total activation of a discrete zone of the synaptic scale. The extension of the minimal effective zone, or perhaps the density of the knobs at a zone of given extension, would depend on the instantaneous threshold of the neuron. Fact ii, however, indicates that no great reduction of the required strength of a local stimulus may be expected from the arrival of impulses to distant knobs.

Subliminal fringe. In the case of a sympathetic ganglion set in activity by electric shocks delivered to its preganglionic trunk, all the fibers of the trunk act as a homogeneous group. Therefore, the only selection that can take place in the ganglion is the number of ganglion cells responding. After a given volley, some ganglion cells will fire while others will remain in the subliminal fringe (Denny-Brown and Sherrington, 1928), the situation being that discussed by Sherrington (1931) on the basis of an illuminating diagram intended to explain the gradation of intensity of motor reflexes. But in the case of the central nervous system, where a state of rest does not seem to be possible, at least not under ordinary experimental conditions, the situation is different; in every instance the fibers having synapses on the neurons constitute several functional groups, and consequently activation of different fibers may result in a change, not only of the number of responding neurons, but also of the channels through which the transmission is effected.

Reflex reversal. This is graphically shown in the diagram of Fig. 2. Fiber *h*—which represents a number of similar fibers—has many synapses on neuron *N*, and a few on neurons a_1 and a_2. Fibers *v.a.* and *v.p.* also represent many similar fibers, each having only a few synapses on cells a_1 and a_2.

In constructing this diagram it has been assumed: (i) that activation of fiber h always results in a discharge of cell N, but does not set up a response of cells a_1 and a_2; (ii) that activation of fibers *v.a.* and *v.p.* fails to set up a response of cells a_1 and a_2; and (iii) that discharge of one of these cells demands stimulation by volleys containing h and *v.a.* or h and *v.p.* impulses. Under such conditions as these it is obvious that if fiber N is activated while fiber *v.a.* is conducting impulses, transmission will take place through neurons N and a_1; but if fiber N is activated while fiber *v.p.* is conducting, the transmission will be established through N and a_2. In each case the motor response will be partly different or even entirely different (reflex reversal). Transmission through N may be said to be a necessary event, it is determined by invariable anatomical moments. But transmission through a_1, or a_2 is conditioned by functional moments of great variability. In short, it is an "optional" transmission.

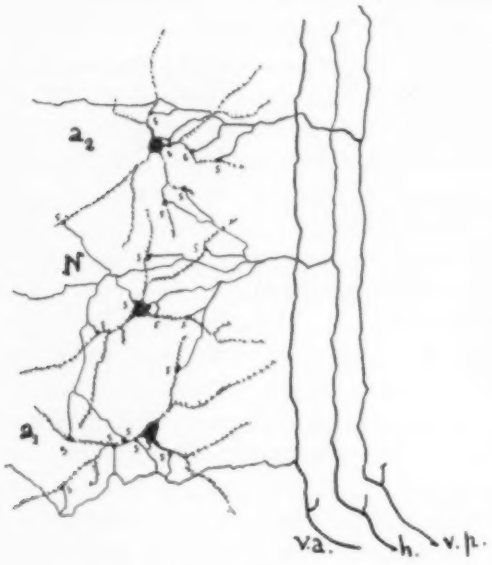

FIG. 2. Diagram explaining the reflex reversal in vestibular reflexes. Further details in text (From Lorente de Nó, 1933*a*).

Experimental conditions for study of synaptic transmission to motoneurons

The motoneurons of the spinal cord, or at least a number of them, can be reached directly by impulses started in a sensory nerve because, as originally described by Cajal (1894), there are collaterals of the posterior columns that reach the motor pools of the anterior horn,* but the motoneurons of the oculomotor or hypoglossal nerves have no synapses with sensory fibers. They can be reached only by impulses started in pools of interneurons. In other words, to reach these motor nuclei, impulses conducted from the periphery by the vestibular, trigeminal, glossopharyngeal, vagus, or any other nerve must cross at least one pool of interneurons, the so-called primary sensory nuclei, which, be it emphatically stated, share with the posterior horn of the spinal cord the property of having a delicacy and complexity of structure not surpassed by any other part of the nervous system, not even by the cerebral cortex. This fact must place the internuncial system of the medulla oblongata at the center of our attention. Also in the case of the spinal cord, consideration of the anatomical conditions at once reveals the extraordinary importance that must be attributed to the internuncial system. Fibers of the dorsal roots without doubt have synapses on motoneurons; but the immense majority of the articulations that they form are synapses with interneurons. If the afferent volley is such that in response to it any motoneuron fires, many interneurons must also have been caused to discharge; furthermore, as fibers of the dorsal roots form much more abundant and denser clusters of synaptic knobs on interneurons than on motoneurons, a response of interneurons must

* Remarkably enough experimental proof, that without facilitation created by a previous afferent volley, these collaterals actually may set the motoneurons into activity, has not been available until recently (cf. Eccles and Pritchard, 1937; Eccles, 1939).

be expected at least after any volley that activates any motoneuron, and presumably even after volleys that are too small to activate any motoneuron.† The internuncial impulses. owing to the short duration of the synaptic delays, are delivered to the motoneurons while the afferent nerve is completing its absolutely refractory period. Consequently there can be no doubt that the effects of a second volley started in the same afferent nerve or of a volley started in another afferent 0.5–0.8 msec. after the first stimulus, must be dependent on the processes that internuncial impulses have created. Under conditions such as these it may be said that a study of the responses of motoneurons after delivery of two shocks

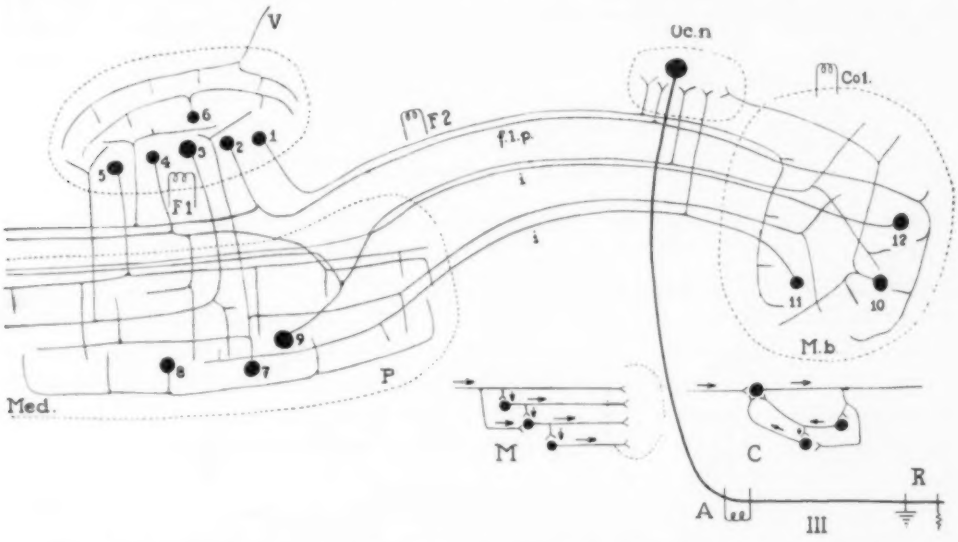

FIG. 3. Diagram of the pathways connecting interneurons among themselves and with the ocular motoneurons. V. vestibular nerve; *1* to *6*, cells in the primary vestibular nuclei; *7*, *8*, *9*, cells in the reticular formation in the medulla (Med.) and pons (P.); *10*, *11*, *12*, cells in the reticular nuclei in the midbrain (*M.b.*); *Oc.n.*, oculomotor nuclei; F1, F2 and Col., positions of the stimulating electrodes. It will be noted that by placing the electrodes on pathways of second order instead of placing them on a peripheral nerve, the passage of impulses through primary nuclei are avoided and the delivery to the motoneurons of large volleys of impulses is insured. The response of the motoneurons can be recorded with electrodes (R) from the trochlear or oculomotor nerve (III). Delivery of a shock to these nerves outside the brain stem through electrodes *A*. causes the arrival of antidromic impulses at the motoneurons.

The diagrams below illustrate the two types of chains, *M*, multiple and *C*, closed, that are found in the internuncial system. In this diagram only pathways of the vestibulo-ocular system have been included; other systems that establish synaptic connections with the ocular motoneurons are arranged according to the same plan. (After Lorente de Nó, 1938 *d*. Fig. 2, with slight additions.)

at variable intervals, or of a train of shocks to afferent nerves or central tracts, is primarily a study of the properties of the internuncial system. Information about synaptic transmis-

† For proofs of internuncial activity in the spinal cord after arrival of dorsal root volleys, cf. the classical paper of Gasser and Graham (1933), the papers of Hughes and Gasser (1934), Hughes, McCouch and Stewart (1936) and the three recent papers of McCouch, Stewart and Hughes (1939). Although not in complete agreement with those investigators, internuncial activity is also postulated by Barron and Matthews (1938). The disagreement is, at least in part, attributable to the fact, that the recording technique used by Barron and Matthews is likely to yield records emphasizing certain potentials at the cost of other potentials (cf. the end of this report).

sion to motoneurons can be obtained solely when the complicating conditions created by the internuncial activity have been carefully considered and eliminated or at least minimized by properly chosen experimental technique.

The oculomotor preparation. An excellent opportunity for the study of synpatic transmission to motoneurons is offered by the reflex arcs that activate the ocular motoneurons, because they include a large fasciculus, the posterior longitudinal bundle and adjacent tracts (Fig. 3, *f.l.p.*), which are known to establish numerous synapses with the motoneurons. Thus, a single shock to these tracts may create a powerful volley of impulses which, after an extremely short conduction time, is delivered to the motoneurons. Extensive studies have been carried out with this system.

II. Experimental Data

Duration of synaptic delay

An important datum has been the determination of the duration of the synaptic delay (Lorente de Nó, 1935*a*). Figure 4 illustrates the time relations of synaptic transmission in the oculomotor preparation. In this experiment single shocks were delivered through electrodes introduced through the anterior colliculus to the level indicated in Fig. 3, *Col.* The responses were recorded from the trochlear nerve shortly after its entrance into the orbit of the eye. Weak shocks (*2*, *3*) produced small discharges of motor impulses which appeared at the recording electrodes after a rather long latency; the discharges increased in size, although their latency did not markedly decrease when the shocks were strengthened up to three times threshold strength (*4*, *5*). But as soon as the shock was made four times the threshold (*6*), the motor discharge showed two distinct waves (*m* and *s*), the earlier one (*m*) with a latency of some 0.7 msec. shorter than that of the second wave (*s*). Further increase of the strength of the shock (*7*, *8*) up to eight times the threshold failed to alter the difference between the latencies of the two responses, but caused an increase of *m* at the expense of *s*. Finally in records not reproduced in Fig. 4 of responses to stronger shocks, the *s* wave failed to appear, obviously because all the fibers of the trochlear nerve were included in the *m* response.

The interpretation of records such as these is at present an easy matter. The shock through electrodes *Col.* (Fig. 3), when weak, stimulated only internuncial axons or cells, and the internuncial impulses thus created, after being delivered to motoneurons, stimulated some of these to discharge new impulses into their axons. The synaptically initiated motor impulses gave rise to the *s* wave. When the shock was strengthened it became capable of stimulating above-threshold elements located at a relatively great distance from the electrodes, *i.e.* motoneurons and motor axons, and these electrically initiated motor impulses gave rise to the recorded *m* wave.

Any of the records with *m* and *s* waves may be used to estimate the duration of the synaptic delay at the motoneurons. The latency, *i.e.* the shock-spike time, of the *m* wave in the recorded responses includes: (i) latency at the cathode of the motor impulses, and (ii) conduction time from the nucleus

to the recording electrode. The latency of the *s* wave includes, (ii) latency at the cathode of the internuncial impulses, (ii) a negligible conduction time to the motor nucleus, (iii) the synaptic delay at the montoneurons, and (iv) conduction time to the recording electrodes. As the latency at the cathode must have been very nearly, if not exactly, the same for motor and internuncial impulses, it is evident that the difference between the shock-spike intervals of both waves measures with sufficient accuracy the synaptic delay at the motoneurons. For example, record *9* shows that the *s* wave is not as synchronous as the *m* discharge, obviously because different motoneurons responded after slightly different synaptic delays, but still the delay was quite constant and for the majority of the responding motoneurons measured about 0.7–0.8 msec. Another important fact illustrated in Fig. 4 is that the synaptic delay cannot be reduced below a certain duration. There are two distinct waves in records *6* to *9*, each with its fixed latency, but there are no discharges at intermediate latencies, *i.e.*, the change in latency for those impulses that passed from the *s* to the *m* wave was not gradual, but step-like.

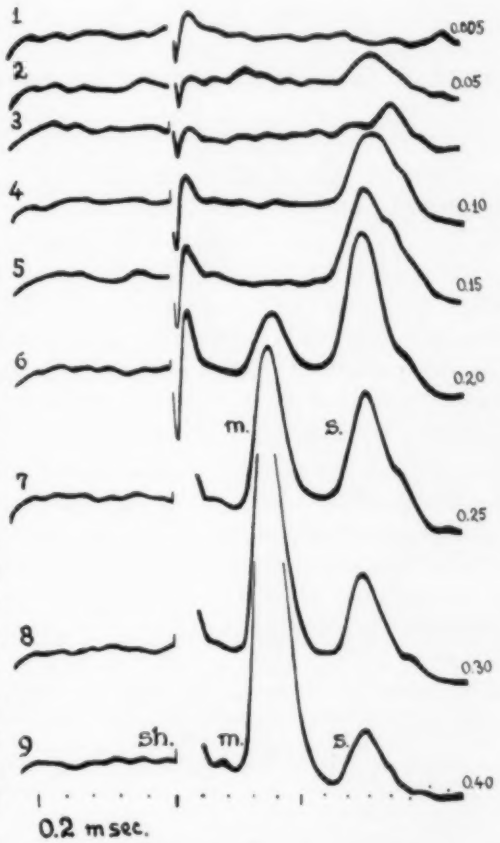

FIG. 4. Oculomotor preparation; responses recorded from the trochlear nerve (Expt. 2-II-36). Stimulating electrodes in position *Col.* (Fig. 3). The numbers on the right side of the records indicate stimulus strength in potentiometric units. *sh*, shock artifact; *m*, spike attributable to direct electric stimulation of motoneurons or motor axons; *s*, spike attributable to synaptic stimulation of motoneurons by impulses initiated by the shock in internuncial axons or somas. The undulations in the records are due to asynchronous impulses of the tonic labyrinthine innervation of the eye muscles.

Limits of variation of synaptic delay. Experiments carried out with a more delicate technique (Lorente de Nó, 1935*d*, *e*, 1938*b*) corroborated this conclusion, and in addition showed that the synaptic delay of motoneurons varies between very narrow limits, from 0.5–0.6 to 0.8–0.9 msec. The low figure is obtained in the case of responses to strong stimuli or responses elicited during facilitation; the high figure when the stimuli are weak or the motoneurons are in a state of partial refractoriness. Therefore, there is in synaptic transmission something similar to the all-or-

nothing law in nerve. After the arrival of impulses at its synapses, the underlying neuron either discharges within that rigidly fixed interval of time or does not discharge unless it is restimulated. The available evidence has been obtained with motoneurons and sympathetic neurons (Eccles, 1936), but there are strong reasons to believe that there are interneurons which, in this respect, behave like motoneurons.

Absolutely refractory period of synaptic arc

In other experiments on the oculomotor preparation the electrodes were placed at some distance from the motor nucleus, for example, at the position F_1 or F_2 (Fig. 3), so that it was possible to use strong shocks without danger of stimulating electrically the axons of the IIIrd or the IVth cranial nerves. With sufficiently strong shocks it is feasible, under favorable conditions, to produce volleys so powerful that all the motoneurons fire in a practically synchronous volley; then it becomes possible to measure the absolutely refractory period of motoneurons, because any response to a second volley initiated by another *F* shock must involve motoneurons that have recovered from absolute refractoriness. It was found (L. de Nó, 1935*b*) that response to a second *F* shock may be produced when the second shock is delivered 0.56 msec. after the first. The conclusion to be drawn from this observation is that in the whole synaptic arc there are no elements (synaptic endings or motoneurons) with an absolutely refractory period longer than that of the presynaptic axons themselves.

The antidromic shock technique. The measurement of the absolutely refractory period of the motoneuron may also be made with the antidromic shock technique (Denny-Brown, 1929) which was so successfully used in studies on the spinal flexor reflex by Eccles (1931) and Eccles and Sherrington (1931*b*, *c*, *e*). In addition, this technique makes it possible to determine, (a) the maximal interval during which the synaptic excitatory process remains at full value, and (b) the temporal course of the recovery of excitability during the relatively refractory period.

The antidromic shock technique is based upon the fact that nerve fibers conduct impulses in both directions. Thus, when a shock is delivered to any point of the motor nerve, it starts an impulse which travels in two directions, centrifugally toward the recording electrodes and centripetally, *i.e.*, antidromically, toward the soma of the motoneuron. In the past there has been some discussion on theoretical grounds concerning the correctness of the assumption of Sherrington (1906) and Eccles and Sherrington (1931*c*) that the antidromic impulse passes through the axon hillock and penetrates into the soma of the neuron. At present, however, there can be no discussion about this point because there is sufficient direct evidence to show that the antidromic impulse enters the soma and there creates changes that have an electrical sign (cf. below, Fig. 14). It may then be taken for granted that upon delivery of a maximal shock to the motor nerve, after the proper conduction time, the soma of all the motoneurons is traversed by an impulse. The experimental evidence presently to be shown demonstrates that after the antidromic impulse the motoneuron passes through a short period, about 0.5 msec., of total unresponsiveness and then through a long period of lowered excitability.

Upper time limit during which synaptic excitatory agent remains at full value. By properly timing the delivery of the antidromic and presynaptic (*F*) shock it is possible to estimate the duration of the interval of time during which the excitatory processes, created by impulses arriving at synaptic knobs, remain at full value. The argument of the experiment is the following.

In a motoneuron that finds itself in a state of absolute refractoriness impulses arriving at synapses should not be expected to create any significant change until after recovery has begun. Thus, if the excitatory agent at the synaptic knob remains at full value for some time after arrival of the impulses, then a discharge will eventually occur; but if the excitatory agent has

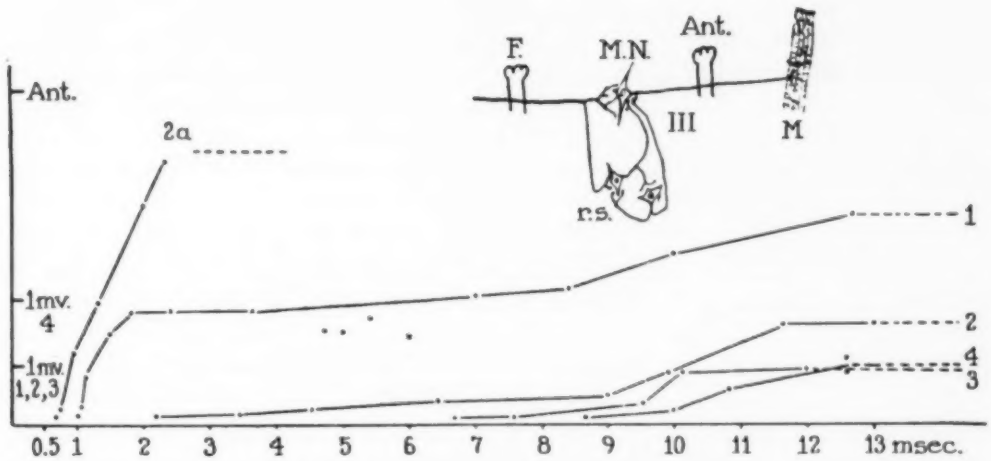

FIG. 5. Oculomotor preparation; responses recorded from the internal rectus muscle (Expt. 9-I-35). The diagram on top explains the conditions of the experiment. *F* and *Ant.* stimulating electrodes in the positions, F_1 and *A* (Fig. 3); *M.N.*, motoneurons; *III*, oculomotor nerve; *M*, muscle; *r.s.*, interneurons of the reticular substance. In the case of curves *1*, *2*, *3* and *4*, one shock through the *F* electrodes caused responses with the height indicated by the broken lines at the right hand side of the curves. The relative strength of the *F* shocks respectively was, in potentiometric units, 0.3, 0.17, 0.06 and 0.05 (note that the amplification was higher in the case of curve *4* than in the case of curves *1*, *2* and *3*). In obtaining curve *2a*, two *F* shocks were used at a 0.6 msec. interval, the first was subliminal for the motoneurons and the second equal to the shock used for curve *2*. When delivered in succession they caused a response with the height indicated by the broken line at the right of curve *2a*. In order to obtain the curves *1*, *2*, *3*, *4* and *2a*, a maximal antidromic shock was delivered that caused a response of the muscle of the height indicated on the ordinate axis (*ant.*) and afterwards at the intervals given in msec. in abscissae the effective *F* shock. The ordinates measure the height of the conditioned synaptic response. Note that while in the case of curve *2a* recovery of height was completed in less than 3 msec., in the case of curve *4* it approached completion at about 12–13 msec., and that while in the case of curve *1* some refractory neurons were capable of responding to the synaptic stimulus 1 msec. after delivery of the antidromic shock, no motoneuron responded to the weaker stimulus (smaller volley) used for curve *4* until after 8.5 msec. after the antidromic shock. (From Lorente de Nó, 1935*c*, Fig. 2.)

a rapid temporal decrement, at the time that the motoneuron recovers from absolute refractoriness, it will be unable to cause the amount of excitation necessary to initiate a new impulse.

In the original report (L. de Nó, 1935*c*) it was mentioned that an antidromic shock delivered 0.43 msec. before the presynaptic (*F*) shock prevented the motoneurons from responding, and that therefore 0.43 msec. was the upper limit of the interval of time during which the synaptic excitatory

process remains at full value. This figure should be corrected, taking into account conduction times in the motor nerve and presynaptic fibers; but it is scarcely worth-while doing so, because the estimated duration at full value of the synaptic excitatory agent is already so small that further reduction would not increase in a significant manner its theoretical significance.

Gradation of intensity of synaptic stimuli. The lowered excitability of the motoneurons after reception of antidromic impulses reveals itself in that the size of the motor discharge in response to a presynaptic volley of given strength becomes smaller during a certain interval of time after delivery of the antidromic shock. A fundamental observation can then be made. Within obvious limits, the response regains its previous size if the presynaptic stimulus (*F* shock) is strengthened (Fig. 5). Since increase of the shock has no other result than to increase the number of impulses delivered at synapses, this result means that while activation of a certain number of synapses is sufficient to reach the threshold of a resting motoneuron, a larger number of synapses must receive impulses in order to reach the higher threshold of a neuron in a state of relative refractoriness. In other words, the results illustrated in Fig. 5 prove the correctness of the conclusion derived by Sherrington (1929) and Eccles and Sherrington (1931*d*) from not so direct an evidence, that the strength of a synaptic stimulus depends upon the number of active synapses. According to the number of impulses that it contains, a synaptic volley may be subliminal, *i.e.*, unable to stimulate any neuron to discharge; liminal, *i.e.*, capable of reaching the threshold of resting neurons; or supraliminal, *i.e.*, sufficient to cause refractory neurons to fire.

Rhythm of discharge of motoneurons. As already stated the presynaptic volley may be made so powerful that it causes the motoneuron to discharge an impulse into its axon immediately after completion of the absolutely refractory period. Usually, however, the rhythm of discharge of the motoneurons is low; in fact, Adrian and Bronk (1929*a*, *b*) reported rhythms of from 5 to 100 impulses per sec. for physiological discharges, and the maximal rate that has been demonstrated with the ocular motoneurons was about 300 impulses per sec. (L. de Nó, 1935*g*). Theoretically, a rhythm of 2000 per sec. is not impossible, and although a continuous discharge at such a rate can scarcely be expected on account of the enormous strength of stimulation that it would demand, the possibility of even only two discharges at a short interval is a fact of considerable importance (cf. later, summation of subnormality).

Recovery cycle of motoneurons

The concept of the subliminal fringe as developed by the Oxford school is based upon an anatomical factor. The afferent fibers to any pool of neurons, before building synaptic knobs, branch out with the noteworthy peculiarity that the territories of distribution of the arborizations of the various fibers overlap partially. It is, therefore, impossible to have a few neurons receiving a liminal number of impulses without other neurons re-

ceiving a subliminal volley. Furthermore, it is impossible to have a discrete number of neurons stimulated liminally without having a number of them stimulated by a supraliminal volley. From this argument it follows that the changes of height of a submaximal synaptic response are a reliable indicator of the instantaneous threshold of the individual motoneurons during the period of depressed excitability which develops after a maximal antidromic shock. Although the law of proportionality is not known, it may be stated that the number of responding motoneurons will be in direct relation to the amount of recovery of excitability of any neuron. Immediately after the

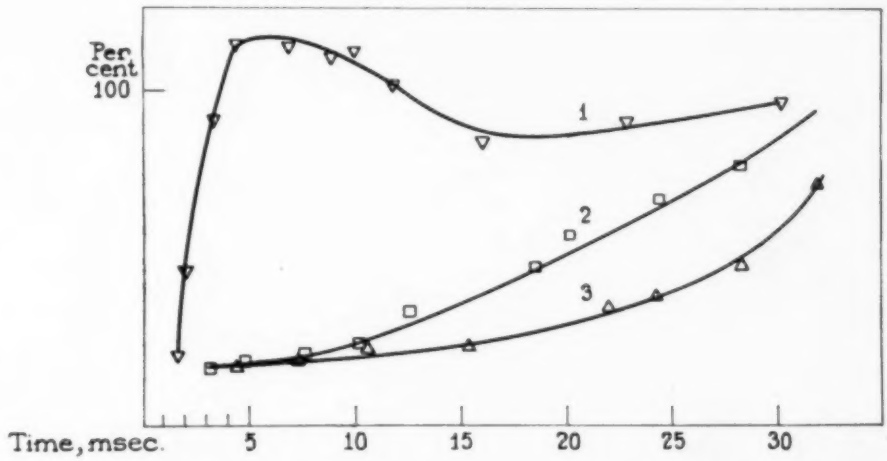

FIG. 6. Oculomotor preparation; responses recorded from the internal rectus muscle (Expt. 12-VI-36). Stimulating electrodes as in Fig. 5. The curves are plots of the height of the testing response against intervals between shocks (abscissae). *1*, recovery curve of the oculomotor nerve, one maximal *Ant.* response conditioning one submaximal *Ant.* response. *2*, recovery of a synaptic response to an *F* shock after delivery of one maximal conditioning *Ant.* shock. *3*, recovery curve of the same synaptic response conditioned by a series of three antidromic shocks at the frequency of 100 per sec., the conditioning interval being measured from the last antidromic shock. (From Lorente de Nó and Graham, 1938, Fig. 2.)

absolutely refractory period only those motoneurons that receive a strongly supraliminal volley respond, while the others remain in the subliminal fringe. As recovery advances, the number of neurons leaving the subliminal fringe and reentering into the response increases; but obviously the unconditioned height will not reappear until all the motoneurons have recovered resting threshold. Thus, the antidromic shock technique allows the mapping of the temporal course of recovery of synaptic excitability in a manner similar to the recovery of electrical excitability of a multifibered nerve in terms of the height of a submaximal testing response elicited at progressively increasing intervals after delivery of a maximal conditioning shock.

The relatively refractory period of motoneurons has been investigated on several occasions (1935*c*, Lorente de Nó and Graham, 1938), and always with essentially identical results. The cycle of recovery (Fig. 6, *2*) includes

a single phase of depressed excitability, which under favorable conditions may be followed during 30–40 msec.; presumably it lasts for a number of additional msec. The asymptotic end of the recovery however prevents the determination of an accurate figure.

Slowed recovery after repetitive activity. It is an important fact that the course of recovery is altered by repetitive activity. Curve *2* in Fig. 6 illustrates recovery after one antidromic shock, while curve *3* represents the course of recovery after three antidromic shocks at the frequency of 100 per

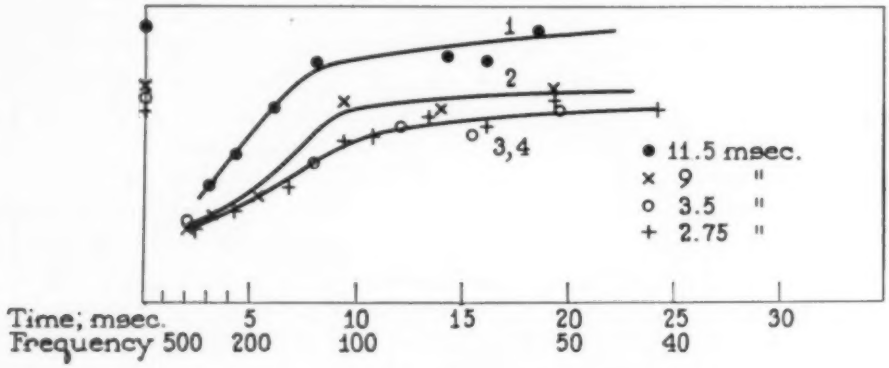

FIG. 7. Oculomotor preparation; responses recorded from the internal rectus muscle (Expt. 12-VI-36). Stimulating electrodes as in Fig. 5. A synaptic response of motoneurons to two *F* shocks in quick succession, both *F* shocks being subliminal for motoneurons when delivered in isolation, is conditioned by two *Ant.* shocks at variable intervals. The interval between the second *Ant.* shock and the testing response was maintained constant (2.75 msec. for curve *4;* 3.5 msec. for *3;* 9 msec. for *2;* 11.5 msec. for *1*). Height of synaptic response plotted against the interval in msec. between the conditioning *Ant.* shocks (abscissae). Height of the synaptic response conditioned by the second antidromic shock only shown on the ordinate axis. The curves, therefore, measure the deficit of response attributable to the increase (summation) of subnormality created by two responses at the indicated frequencies, in relation to the subnormality created by one response. Note that for frequencies of response between 40 and 100 per sec. the increase in subnormality is slight, but for higher frequencies, from 100 to 500 per sec., the increase rapidly grows with the frequency. Moreover, the subnormality after two responses at the frequency of 500 per second remains practically unchanged for 11 msec. (From Lorente de Nó and Graham, 1938, Fig. 5.)

sec. It will be noted that although the early part of the recovery curve shortly after the absolutely refractory period is essentially the same in both cases, the later part of the curve is considerably lowered by repetitive activity. This finding resembles what in the case of nerve is called summation of subnormality (Gasser, 1935). It is a process of considerable theoretical significance because it may play the rôle of the "fatigue" of the neuron, so often assumed, but never experimentally demonstrated in classical neurophysiology. Subnormality, be it distinctly understood, is a special kind of fatigue. It may prevent the response to weak stimuli, but does not prevent the response to strong stimuli; and when a response is elicited, at least in blood-

perfused mammalian nerves, it has the normal size and is conducted at the normal rate (Graham and Lorente de Nó, 1938). Intense and enduring activity of nerve may be followed by a subnormality so strong that it may be called true fatigue; in this state of depression the conduction rate (Gasser, 1935) and even both the conduction rate and height of response (Gerard and Marshall, 1933) may be depressed; but according to the observations of Graham and Lorente de Nó (1938) the amount of activity necessary for that to occur is so large that it practically lies beyond the limits to be expected in physiological functioning of the central nervous system.

As in nerve (Gasser, 1935), the summation of subnormality in motoneurons has the important property of being dependent, not so much upon number, as upon frequency of impulses. In Fig. 7 it is seen that with the oculomotor neurons frequencies of less than 100 impulses per sec. produce a mild summation of subnormality, while higher frequencies cause a marked summation; the more so, the higher the frequency. In fact, as few as two responses at a frequency of 500 per sec. create such a strong subnormality that the response of the motoneurons is maintained at practically the level it has immediately after the absolutely refractory period during a considerable number of msec. Only very powerful stimuli can then produce a response. The significance of this fact has been discussed elsewhere (1938*d*).

Differences between recovery of motoneurons and motor axons. A striking feature of the recovery cycle of the synaptic excitability of the motoneurons (Fig. 6, *2*) is that it does not parallel the cycle of recovery of electrical excitability of their axons (Fig. 6, *1*). It is true that subnormality lasts in the case of both soma and axon for apparently the same length of time, but in the cycle of the axon there often is a period of supernormal excitability which has no equivalent in the cycle of the synaptic excitability of the soma. Supernormal excitability of the axon does not develop in every blood-perfused nerve (Graham and Lorente de Nó, 1938); but even then the contrast between both cycles is striking, because while the axon recovers 95 per cent or more of its electrical excitability in 4 or 5 msec. after conduction, the synaptic excitability of the motor nucleus at that time is still nearly at the same low level as immediately after the absolutely refractory period. The significance of this divergence of the recovery cycles, which also applies to stimulation of nerve by peripheral sensory endings and by electric shocks (cf. Gasser, in this Symposium), will be discussed later in relation to a model of synaptic transmission.*

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* In this connection it must be mentioned that the speed of conduction of a nerve does not become greater during the supernormal phase when the electrical excitability is enhanced, nor smaller during the subnormal phase when the electrical excitability is depressed (Graham and Lorente de Nó, 1938). In other words, changes in electrical excitability may fail to make themselves apparent in modifying restimulation of the nerve by its own action current. Undoubtedly, the processes underlying supernormality and subnormality produce changes of more than one parameter of excitability.

Facilitation of motoneurons and the c.e.s.

In any oculomotor preparation in which a single shock to the posterior longitudinal bundle results in a discharge of motoneurons, the size of the response increases with the strength of the shock, *i.e.*, with the number of impulses in the presynaptic volley. Occasionally the response grows up until all the motoneurons are engaged, but usually the maximal response to a single shock of any strength includes only a fraction of the total number

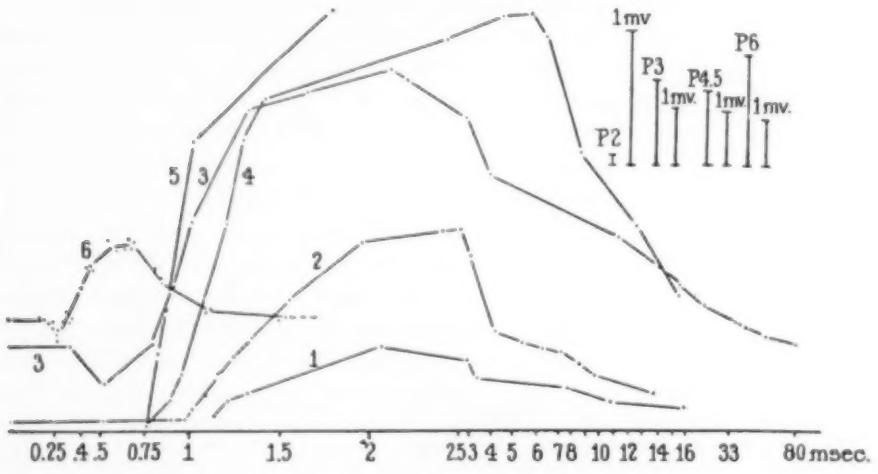

FIG. 8. Oculomotor preparation; responses recorded from the internal rectus muscle. The ordinates measure the height of the response to an *F* shock when conditioned by another *F* shock at the interval indicated in msec. in the abscissae.

1–5. (Expt. 18-XI-34). Vestibular nuclei destroyed and also a transverse section in pons as in Fig. 15, *I* in Lorente de Nó, 1933*b*, Fig. 16 in that paper shows that as a consequences of the lesion the vestibular after discharge was enormously prolonged; a similar process may explain the unusually long duration of facilitation in curves *1* to *5*. The testing *F* shocks alone produced responses with height P_2, P_3, $P_{4,5}$ (cf. upper right corner). The ratio of strengths of the conditioning and testing shocks were: *1*, 40/100; *2*, 50/100; *3*, 40/100; *4*, 160/100; *5*, 120/100. In *4* the testing shock was just maximal, in *5* supramaximal (125/100).

6. (Expt. 19-XI-34). Intact medulla. Conditioning shock just above threshold. Testing shock 250/100 larger. Note facilitation starts at a 0.4–0.43 msec. interval between shocks, and lasts for about 1 msec. while in *3* it lasted for 80 msec. 1 mv. = one millivolt. (From Lorente de Nó, 1935*d*, Fig. 1.)

of motoneurons. In such cases it is possible to obtain a further increase, indeed it is often possible to cause all the motoneurons to fire almost synchronously, by delivering to the posterior longitudinal bundle, instead of one, two successive shocks of which the first or conditioning shock may be subliminal, *i.e.* so small that the volley of impulses produced by it does not reach the threshold of any motoneuron. It is obvious that the conditioning volley, whether liminal or subliminal, creates some process which "facilitates" the stimulation of motoneurons by the second or testing volley. The temporal course of facilitation is best represented by "facilitation curves"

obtained by plotting the size of the testing response which is proportional to the number of responding neurons against the interval between shocks. A number of facilitation curves obtained with the oculomotor preparation have been reproduced in Fig. 8 and 9 (cf. also Fig. 13).

The concept of the central excitatory state (*c.e.s.*) Facilitation in the oculomotor preparation is entirely comparable to the well-known phenomenon

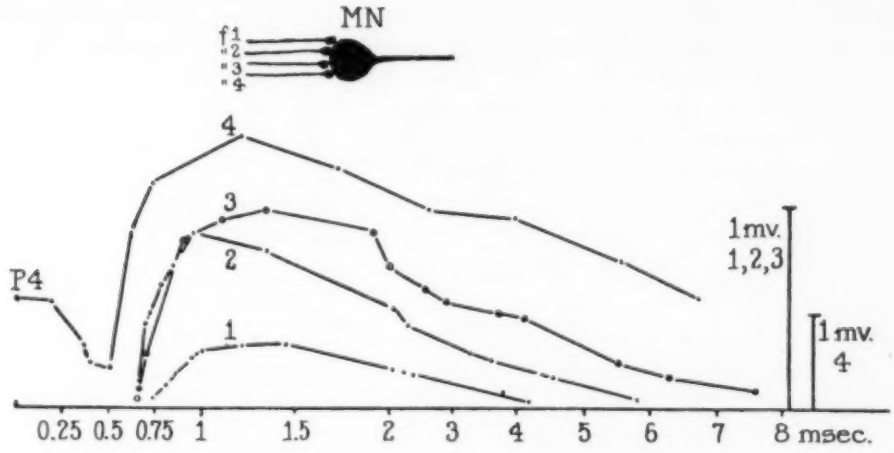

FIG. 9. Oculomotor preparation; responses recorded from the internal rectus muscle. Height of the response to the testing *F* shock (ordinates) plotted against the interval in msec. by which it followed a conditioning *F* shock (abscissae).

1, 2, 3. (Expt. 3-XII-34). Vestibular nuclei and reticular substance in medulla and pons destroyed. Both *F* shocks subliminal when delivered in isolation. Ratio between strengths of conditioning and testing shocks: *1*, 50/100; *2*, 60/100; *3*, 160/100.

4. (Expt. 7-I-35). Intact medulla. Subliminal conditioning shock 60/100 of the testing shock. P_4, height of the unconditioned testing response. The diagram on top explains the depression of the testing response in curve *4* at intervals between shocks of more than 0.25 and less than 0.5 msec. The conditioning shock is supposed to have stimulated fiber *f1* and the testing shock, when delivered in isolation, fibers *1* to *4*. When both shocks were delivered in succession at intervals of less than 0.5 msec., the testing shock stimulated fibers f_2, f_3 and f_4 only, because fiber f_1 had responded to the conditioning shock. Therefore, as soon as the detonator action of impulse f_1 began to decay, the response to the testing shock was depressed. (From Lorente de Nó, 1935*d*, Fig. 2.)

of the facilitation of reflex responses in the spinal cord and should be explained in similar terms. A suggestive explanation of facilitation was offered by Sherrington (1925; cf. Fulton, 1926; Bremer, 1930; Eccles and Sherrington, 1931*d*). Facilitation was believed to depend upon the creation in the motoneurons of a certain process called *c.e.s.* (central excitatory state) characterized: (i) by its being enduring, *i.e.*, its being dissipated at a relatively slow rate, and (ii) by its being capable of summation. In certain respects *c.e.s.* was compared with Lucas' (1917) local excitatory process in nerve. Each subliminal volley of impulses would create a certain amount of *c.e.s.* and summation of successively produced quanta would eventually yield the amount necessary to reach the threshold of the motoneuron and

set up a discharge. According to this assumption, which has been one of the most fruitful working hypotheses ever introduced in neurophysiology, facilitation would take place within the motoneurons, or in general, within the individual neurons.

Insufficiency of concept of c.e.s. for complete explanation of known facts. The creation in subliminally excited neurons of a process of the nature assumed for the *c.e.s.* has never been disproved, but recently it has become apparent that the hypothesis of *c.e.s.*, as originally presented, is not sufficient to explain newly acquired facts. In the first place, if effective excitation were

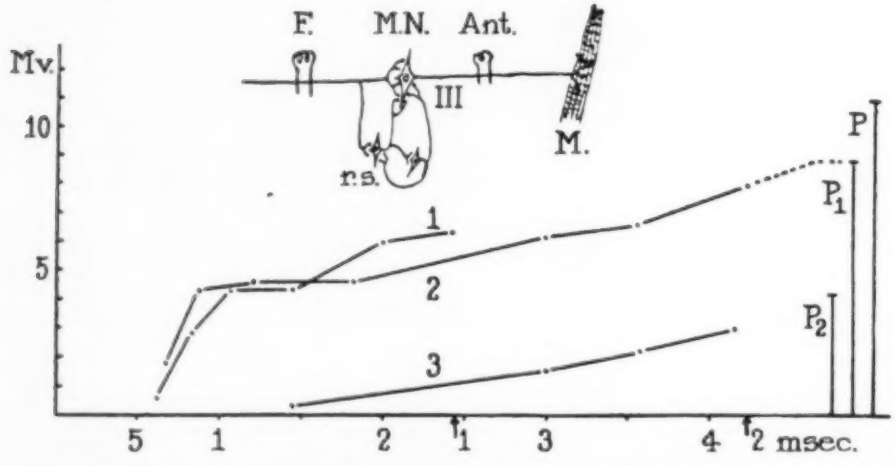

FIG. 10. Oculomotor preparation; responses recorded from the internal rectus muscle (Expt. 7-I-35). Stimulating and recording electrodes as in Fig. 5. The curves are plots of the response to a testing *F* shock with unconditioned height P_2, when this response was conditioned by a maximal antidromic shock (*3*) or by a maximal antidromic shock and a conditioning *F* shock (*1*, *2*). The response to the testing *F* shock, when conditioned only by the conditioning *F* shock, was facilitated and its height became P_1. The fixed interval between both *F* shocks is indicated by arrows *1* and *2* below the abscissa axis. The abscissae measure the interval between the antidromic and the second *F* shock. Therefore, the antidromic shock was delivered first between both *F* shocks, then simultaneously with the conditioning *F* shock and finally before this shock. Comparison of curve *3* with curves *1* and *2* show that the antidromic shock at no moment prevented the creation of or destroyed all the facilitation that had already been created. P_1, height of the response to the antidromic shock (From Lorente de Nó, 1935*c*, Fig. 4).

due to summation of successively produced quanta of *c.e.s.*, the synaptic delay could not have the rigidly fixed limits that it has, especially not its relatively long minimal duration. On the contrary (cf. Eccles and Sherrington, 1931*a*), the synaptic delay should vary between wide limits and should be reduced to almost nothing in facilitated responses. Then, according to the *c.e.s.* hypothesis, the subliminal changes underlying the state of facilitation should be destroyed by the all-or-nothing response initiated by the entrance into the motoneuron of an antidromic impulse. In fact, however, facilitation is not destroyed by the entrance of an antidromic impulse into the motoneuron (Lorente de Nó, 1935*c*).

Facilitation created during absolute refractoriness and facilitation persisting after an all-or-nothing response. While delivering a maximal antidromic shock to the motor nerve at various intervals before the conditioning shock or between the conditioning and the testing shock (Fig. 10) it was found (1935c) that facilitation may be created when the conditioning volley finds the motoneurons in a state of refractoriness, even of absolute refractoriness; and also facilitation was not destroyed by the entrance of an antidromic shock into the soma of the motoneurons, although, as already indicated, the antidromic impulse by initiating an all-or-nothing response of the soma must have wiped off any enduring change initiated by the subliminal conditioning volley. The conclusion drawn from this experiment was that the antidromic volley had no effect on facilitation except by its depression of the excitability of the motoneurons. This conclusion might have been somewhat too radical because the experiment does not prove in a conclusive manner that enduring subliminal changes were not produced in the motoneurons by the conditioning volley; but the conclusion was strongly supported by the absence in curve *2* in Fig. 10 of an apparent discontinuity at the point of simultaneous delivery of the antidromic and the conditioning shocks. Indeed, in view of curve *2* in Fig. 10 it must be stated that if an enduring subliminal change had been created in the motoneuron by the conditioning volley, its participation in maintaining facilitation was small in relation to the effect of some other agent for facilitation which could not be reached by the antidromic impulse and therefore was located outside the motoneuron.

Extracellular agents for facilitation. Several extracellular agents may be considered. First, it may be assumed that a chemical agent is released by the synaptic endings, and remaining outside the motoneuron, is not affected by the antidromic impulse. In former times, and in relation rather to long lasting responses (after discharge) than to facilitation, the possibility of enduring chemical changes at the synapses was considered by Fulton (1926), who later (1938), after taking into account newly acquired evidence, has expressed the view that, as the arguments underlying the original hypothesis have lost a great deal of their force, long-lasting excitation can be explained chiefly in other terms. Rosenblueth (1934) and Forbes (1934) were strongly in favor of chemical agents; but, it seems that these views too have become largely superseded (cf. the views held by Cannon and Rosenblueth, 1937). The problem of extracellular agents for facilitation has, however, recently acquired a new aspect, because Barron and Matthews (1938) have developed a theory which, although considering chiefly unspecific ions, still leads to the assumption of extracellular agents for facilitation that, with respect to the temporal course of their action, could be compared with chemical agents. Barron and Matthews believe that long-lasting differences of potential between the synaptic endings and the parent fibers set up changes of ionic concentration in the neighborhood of the soma of the nerve cells, thus creating in them a certain degree of depolarization which eventually may lead to a rhythmic discharge of impulses.

Extracellular agents of this type would create facilitation and would not be accessible to antidromic impulses. Thus, its existence cannot be excluded on the basis of the experiment of Fig. 10. Moreover, environmental changes, chemical or only of concentration of unspecific ions, must now be considered as possible agents for facilitation because there is factual evidence brought forward by Dusser de Barenne, McCulloch and Nims (1937; see also Dusser de Barenne and McCulloch, 1939) that during activity changes take place in the nervous system which make themselves evident by changes of pH and lead to alterations of excitability that parallel those known to occur in nerve when the pH of the medium is varied (Lehmann, 1937). In this connection the experiments of Gerard and collaborators (cf. Gerard, 1936) must also be cited, as reference must also be made to important work on the effect of chemical agents in the response of sympathetic ganglia (cf. Bronk, in this Symposium).

There can be no doubt that changes in the environment of the nerve cells, no matter what might be their origin, will alter the excitability of the neurons. Thus, these changes may eventually result in a lowering of threshold and facilitation of the responses to a given stimulus. The question is, therefore, whether environmental changes are the only mechanism underlying facilitation and, if not, whether they are the primary mechanism.

Impossibility of explaining facilitation solely in terms of chemical change. Proceeding along a line of argument similar to that employed by Eccles and Sherrington (1931*e*) in their discussion of inhibition by enduring chemical agents, the following remarks must be made (cf. Lorente de Nó, 1936). Facilitation by chemical agents released by impulses at the synaptic endings should follow a temporal course of quite definite configuration; in fact, the chief argument adduced by Rosenblueth (1934) in favor of chemical agents was derived precisely from a comparison of the temporal course of central responses with the course of peripheral responses believed to be due to chemical reactions. Thus, if facilitation were due *only* to changes created by the conditioning volley in the neighborhood of the motoneurons, then no essential differences in the course of facilitation should be observed in different preparations, nor in the same preparation when conditions are altered at points distant from the facilitated motoneurons. There is no doubt that suitable and permissible assumptions could be made in order to interpret any of the curves in Fig. 8 and 9 in terms of the creation and dissipation of chemical agents or of changes of ionic concentration in the environment of the neurons: but there is also no doubt that the assumptions made, for example, for the case of curve *1*, Fig. 8, cannot apply to curve *6* in the same Fig. 8. Nor can any assumption capable of accounting for curve *3* in Fig. 8 explain the curves in Fig. 13. Again, assumptions made for the facilitation of motoneurons of the oculomotor nucleus would not be suitable for the motoneurons of the hypoglossus nucleus, etc. The differences in the time of onset, rate of ascent, position of maximum height, duration and rate of descent found in the facilitation curves are so enormous that they demand at least

the additional assumption of some other mechanism of facilitation. In other words, these great differences indicate that whether chemical changes took place in the neighborhood of the motoneurons immediately after arrival of the conditioning volley or not, and whether the changes persisted for some time and may account for part of the facilitation or not, still some other extracellular mechanism was operative in maintaining facilitation. The additional factor has proved to consist of subliminal stimulation of motoneurons by a long lasting stream of internuncial impulses (internuncial bombardment).

Restimulation of motoneurons by subliminal volleys of internuncial impulses. The idea that interneurons reenforce the transmission of the current of "nervous energy" effected by simple and direct pathways was originally stated by Ramón y Cajal (1901; 1911, p. 150) who, from his own anatomical discoveries, concluded that the interneurons are arranged in parallel chains superimposed upon the simple two-neuron arcs. Several illuminating diagrams may be found in Cajal's monumental work (1911, Fig. 103 and 104; reproduced in Lorente de Nó, 1933*b*, Fig. 2). In contemporary neurophysiology the concept of long-lasting stimulation (after discharge) by impulses delayed during their passage through internuncial synapses was introduced by Forbes (1922), and elaborations of this concept were suggested by Bremer and Rylant (1926), Ranson and Hinsey (1931), Forbes, Davis and Lambert (1931), the present author (1932) and others. Eccles and Sherrington (1931*c*, *e*), in order to account for experimental results obtained in studies on after-discharge and sustained inhibition of the flexor reflex, also postulated the existence of a bombardment of motoneurons by internuncial impulses. In this connection it might not be impertinent to mention that, no matter how much complexity of structure could be attributed to the nervous system, still the possibility of the postulated internuncial activity was not established* (cf. Eccles, 1936, p. 395) until it was demonstrated that the internuncial circuits described by Ramón y Cajal (1911, Fig. 103 and 104) were not peculiar to parts of the nervous system of highly specialized structure, *i.e.*, the cerebral and cerebellar cortices, as he had stated (1911, p. 150–151) but with few exceptions are present everywhere in the central nervous system (Lorente de Nó, 1933*b*). Experimental studies (*Idem.*, 1926, 1928; summary 1931) had previously brought to light a number of facts which, as it seems (cf. Sherrington, 1934), conclusively demonstrated the participation of the internuncial system in the establishment of motor reflexes. One of these facts was (1928, p. 105–107; cf. 1933*b*, Fig. 18) that the duration of the response to a given peripheral stimulus depends upon the activity of internuncial relays. Thus, it was not difficult to arrive at the conclusion (1935*c*, Fig. 2, 1935*d*) that facilitation of motoneurons is the result of continued bombardment of the motoneurons by internuncial impulses arranged in volleys of subliminal density, which summate with the impulses initiated by the testing volley.

* Reference must be made to the review, that Forbes published in 1934 (p. 188–190).

Period of effective summation of impulses arriving at different synapses. Before attempting an analysis of the internuncial activity that underlies facilitation and after-discharge, it is necessary to determine the duration of the period of effective summation of nerve impulses delivered to different synapses on the motoneuron. For this purpose two methods have been used: (i) fractionation of a synchronous volley of impulses into two volleys delivered in succession at different intervals (1935*d*, Fig. 2, *4*), and (ii) delivery to the motor nucleus of two volleys carried by different fibers (1935*e*). Experiments in which the summation of subliminal volleys of impulses with induction shocks were investigated (1935*f*) led to similar conclusions.

The conclusion may be expressed in the following statement. When two volleys of impulses are delivered to different synapses on a motoneuron, the statistical chances of effective summation are greatest if the volleys are delivered simultaneously or at intervals of less than 0.15 msec. They decrease rapidly when the volleys are separated by progressively increasing intervals of time, because some impulses fail to summate when they have arrived at intervals of over 0.15–0.2 msec. Finally, the chances of effective summation disappear when the separation between volleys becomes as small as 0.5 msec.

Possible existence of a second period of lowered threshold of motoneurons after subliminal synaptic stimulation. Eccles (1936, 1937) working on the transmission of impulses through the superior cervical ganglion obtained, with the fractionation method and the double volley technique, results similar to those reported with the ocular motoneurons and therefore concluded that the nerve impulse upon its arrival at the synapse creates an excitatory process of extremely brief duration, the "detonator" response which, if sufficiently strong, after completion of the synaptic delay leads to the discharge of a new impulse by the ganglion cell. The detonator action of the nerve impulse would be identical with what the present author called "synaptic excitatory process" (1935*c*, *d*; cf. Eccles, 1939, p. 368). In addition, Eccles made the important discovery of a *second* period of lowered threshold of the ganglion cell, which would develop after dissipation of the detonator action. In order to account for the second period of lowered threshold, Eccles assumed the production in the neuron of a certain change which he termed *c.e.s.* There is, however, an essential difference between Eccles' concept and the original concept as developed by Sherrington (1925) and by Eccles and Sherrington (1931*d*). According to Eccles, *c.e.s.* should only modify the threshold of the ganglion cell without ever reaching sufficient strength to initiate the discharge of an impulse. The initiation of a new impulse would demand the arrival of new impulses to synapses and the creation of their detonator actions.

Spatial and temporal summation

Eccles' observations are of fundamental importance because they reopen the question of how far facilitation may be attributed to protracted changes in threshold in individual neurons. The question is indeed an essential one. If nerve impulses arriving at synapses should develop only detonator actions which are no longer in duration than the absolutely refractory period of presynaptic fibers, then temporal summation of impulses arriving in succession through the same fiber would be impossible, and in the nervous system no other type of summation could exist than the spatial summation of impulses arriving simultaneously or within a short interval of time through different synapses. But if subliminal impulses should produce a second phase of lowered threshold, then temporal summation of impulses conducted in succession by the same fiber would occur.

Second phase of summation in different structures. In view of the results obtained by Eccles with ganglion cells, the temptation is great to assume the development of *c.e.s.* in subliminally stimulated motoneurons. It is indeed great because processes comparable to those observed in the ganglion cells have been demonstrated for other structures. The following recent observations need mention here. In the partially curarized neuromuscular junction of the frog one impulse may fail to cross the junction, but it creates a certain enduring process, which enables a second impulse to cross the junction (Bremer, 1930; Bremer and Homès, 1932). An impulse may fail to cross an anodal block in nerve, but it causes an enduring change which enables a second impulse to cross the block (cf. Erlanger, in this Symposium). Stimulation of an adequately treated nerve by a train of shocks at low frequency results in a progressive increase of the number of responding fibers (recruitment), which undoubtedly proves the existence of a long period of lowered threshold in nerve fibers subliminally stimulated by electric shocks (Gasser, 1938). In agreement with this conclusion a second phase of enhanced excitability may be directly demonstrated in blood-perfused mammalian nerve by stimulation with a train of subliminal shocks of high frequency. After the last shock of the series the nerve successively passes through: (i) a short lasting period of lowered threshold, which corresponds to the period of local summation of subliminal shocks of Lucas and Adrian (1917); (ii) usually postcathodal depression, but sometimes only return to normal excitability; and (iii) a long-lasting second period of lowered threshold (Lorente de Nó, quoted by Gasser, 1938). Similar effects, except for the lack of postcathodal depression, may be observed after a single shock in anodally polarized nerves (Blair, 1938a).

With data such as these it is not surprising that the consideration of a second period of summation for the interpretation of facilitation of central neurons has been demanded by a number of investigators (Eccles, 1936, 1939; Gasser, 1938). It is even understandable that, despite the absence of conclusive evidence, the demand has been made in emphatic terms (Bremer and Kleyntjens, 1937).

Failure to demonstrate second phase of summation in motoneurons. It has been impossible, however, to demonstrate in a convincing manner the development of a second phase of lowered threshold in subliminally stimulated ocular motoneurons. This lack of success does not prove that a second phase of summation did not develop; as a matter of fact, it proves only that the lowering of threshold due to such a process is small in relation to the change in threshold produced by alteration of the intensity of the internuncial bombardment, and therefore cannot be demonstrated if a variation in the strength of the internuncial bombardment takes place. There is experimental evidence in support of this conclusion.

The records in Fig. 11 are an excellent illustration. In this experiment two different electrodes were used to create volleys of presynaptic impulses. One shock was delivered through electrodes *F* (Fig. 3) and the other through electrodes *Col.* (Fig. 3). In isolation

the weak *F* shock set up responses (Fig. 11, *3*, *5*, *18*) composed of several waves, the first one due to impulses set up in fibers of the posterior longitudinal bundle, and the following responses due to impulses delayed during their passage through internuncial neurons (cf. 1938*d*, Fig. 3). The *C* shock, however, caused a response composed of an almost synchronous spike, which was followed by a temporary cessation of the labyrinthine tonic

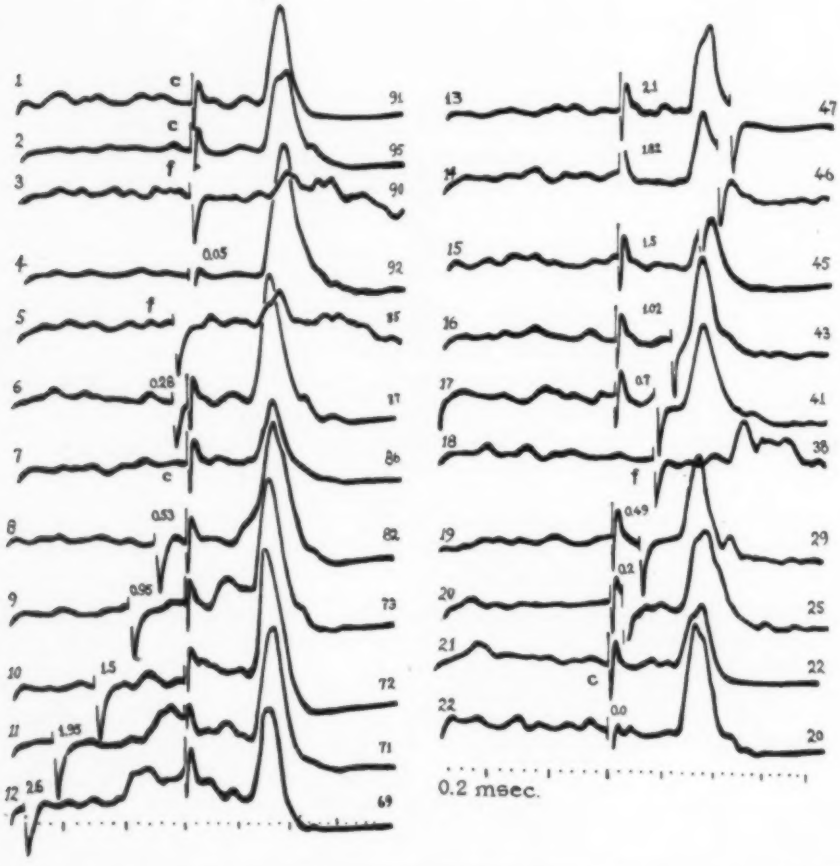

FIG. 11. Oculomotor preparation; responses from the trochlear nerve. (Expt. 2-II-36). Two stimulating electrodes in positions F_1 and *Col.* (Fig. 3). The responses to the *C* and *F* shocks in isolation have been reproduced in records *1*, *2*, *3*, *5*, *7*, *18* and *21*. There was a certain variation of height of the *C* response due to the discontinuous character of the tonic labyrinthine innervation, the response being of course larger when the *c* impulses happened to coincide with a large internuncial volley. Record *1* reproduces the largest observed response to a *C* shock in isolation. The numbers on the right hand side of the records indicate the order in which they were obtained; between each two consecutive records there was an interval of two seconds. For records *4* to *12* the *F* shock preceded the *C* shock at the interval indicated in msec. on the records. For records *13* to *22* the *C* shock preceded the *F* shock. Time in 0.2 and 1 msec. below (from Lorente de Nó, 1938*d*, Fig. 7).

discharge usually present in electrograms of the eye muscles or their nerves. As the spike included only a fraction of the trochlear motoneurons, this silent period cannot be ascribed to refractoriness of motoneurons. The *F* and the *C* shocks may have in part stimulated the same fibers, but there can be no doubt that they also stimulated different fibers, because when delivered simultaneously (*22*) more motoneurons discharged than when either

shock was delivered in isolation. When the *F* preceded the *C* shock, the response to the latter was facilitated (*4* to *12*), the period of facilitation lasting through the duration of the *F* response. The facilitation curve in this case did not show a trough around the 0.5 msec. interval. Apparently the weak *F* shock set up impulses after various latencies and the *c* impulses always met in the nucleus some *f* impulses with which they could summate.

The results were entirely different when the *C* preceded the *F* shock. At an interval of 0.2 msec. (*20*) the late waves of the *F* response were greatly reduced and even the early wave, which was superimposed upon the descending phase of the *C* response, was reduced in size. At the 0.49 msec. interval (*19*) the *F* response was reduced to a small initial wave. At greater intervals, up to 4 msec., it was totally abolished, as was also the case when the *C* response included only a small number of motoneurons (*15*). The *F* response began to reappear at an interval of about 4 msec. between shocks and up to the last interval studied, 5 msec., it did not show signs of being facilitated.

Since there was response to the *F* shock at the 0.2 and 0.49 msec. intervals when the *C* response was large and there was no *F* response at longer intervals (*15*) when the *C* response happened to be small, the absence of *F* response at intervals of over 0.5–0.7 msec. cannot be ascribed to the failure of the *F* shock to set up impulses in presynaptic axons that had been made refractory, nor can it be ascribed to refractoriness of motoneurons included in the *C* response. The only possible explanation would be the following. The *C* shock created a volley of impulses (*c*) which was delivered to the motoneurons, and at the time that the motoneurons were responding a temporary cessation of the internuncial bombardment, responsible for the tonic labyrinthine innervation, took place. This interruption of the internuncial bombardment resulted in the silent period in the electrogram of the trochlear nerve. The *c* volley stimulated a number of motoneurons above threshold and other motoneurons subliminally. As long as the synaptic excitatory processes created by the *c* impulses were able to summate with those created by the *f* volley, a number of motoneurons fired in response to the *F* shock and produced an *f* wave in the recorded response; but as soon as these detonator actions were dissipated, since the tonic background of excitation had been suppressed, the *f* impulses remained ineffective. This happened about 0.5–0.7 msec. after delivery of the *C* shock. Afterwards no response to the *F* shock was observed until the tonic labyrinthine innervation was reëstablished. Therefore, the conclusion is unavoidable that if the *c* impulses had produced in subliminally stimulated motoneurons a second period of lowered threshold, or had resulted in the release of chemical agents, or in changes of ionic concentration in the environment of the motoneurons, all these effects were weaker than the rise in threshold due to the cessation of the indeed mild internuncial bombardment responsible for the labyrinthine tonus. Hence there resulted the temporary inhibition (extinction) of the *f* response.

Upper limit of lowering of threshold possibly attributable to c.e.s. or environmental changes with brief stimulation. If it were permissible to apply to motoneurons quantitative results obtained with motor axons, then it could be stated that the lowering of threshold that might be expected during the second phase of summation (*c.e.s.* of Eccles) is no more than 4–5 per cent of the resting threshold, because this is the maximal increase in excitability ever observed during the second phase of summation created in the trochlear nerve by a rhythmic series of subliminal induction shocks (unpublished experiments). A similar upper limit can be estimated from the results of other unpublished experiments in which the excitability of the motoneurons was tested with induction shocks. Internuncial bombardment easily produced a lowering of the electrical threshold of motoneurons amounting to 50 per cent or more of the resting value.

The ability of the internuncial bombardment to mask the effect of other possible agents for facilitation is also shown by other observations. Facilitation attributable to a second phase of summation of the motoneurons, judging by what is known from ganglion cells, neuromuscular junctions and

nerve fibers, should start rather late and have a gradual onset. For example, with the trochlear nerve, the second phase of summation does not start earlier than 1 msec. after delivery of the last conditioning shock. A similar late and gradual onset should be expected for facilitation due to changes in ionic concentration in the environment of the neuron (Barron and Matthews, 1938). But facilitation due to internuncial bombardment begins early, *i.e.* as soon as synaptic delay at interneurons has been completed, and under favorable conditions it may be expected to have a sudden onset, reaching maximal value in a fraction of a millisecond. An early and sudden onset is frequently observed (Fig. 8, 9, 13), and since at intervals between shocks of 0.43–0.75 msec. facilitation can scarcely be attributed to anything but internuncial bombardment (*i.e.* to instantaneous summation of detonator actions), it is obvious that the internuncial bombardment is a powerful agent for facilitation. Consequently, if the internuncial bombardment is maintained, the effect of other facilitatory mechanisms will scarcely be detectable until the bombardment ceases (cf. Dusser de Barenne and McCulloch, 1939, p. 334).

The conclusion to be drawn from the preceding remarks is, therefore, the following. Whether in subliminally stimulated motoneurons after dissipation of the detonator actions a second phase of lowered threshold develops, and whether in the immediate neighborhood of the motoneurons chemical agents are released, or changes in ionic concentration take place, which result in a lowering of threshold of the motoneurons,—these are still unsolved questions. In view of the available evidence it seems likely that processes such as these will be demonstrated with new techniques, but it must be expected that the lowering of threshold attributable to them will amount to a few per cent of the resting threshold, and therefore will be small in relation to the great lowering that may be produced by the detonator actions of the impulses of the internuncial bombardment (cf. Fig. 8, 9 and 11). For this reason, in theoretical arguments intended to represent only a first approximation, it is permissible to explain facilitation solely in terms of the short synaptic excitatory processes (detonator actions) created by the impulses of the internuncial bombardment (cf. Lorente de Nó, 1938*d*, p. 221 and 228). The possible existence of other agents for facilitation will have to be considered from the first moment in those cases in which interneurons are not present, as for example, in the cases of sympathetic ganglia, the dentate nucleus of the cerebellum, the nuclei of Goll and Burdach, the superior olive, etc. Unfortunately, except for studies on sympathetic ganglia, the course of facilitation in relays of this type has not been investigated. On the other hand, it must be considered that enduring intra- or extracellular changes, especially cumulative changes, will not only lead to modifications of the internuncial bombardment, but will also be the only factors capable of explaining facilitation or inhibition (extinction) remaining after cessation of enduring bombardments of neurons by nerve impulses (cf. Dusser de Barenne and McCulloch, 1939; Bronk, in this Symposium).

Mechanisms underlying internuncial bombardment

If prolonged excitation is maintained in the neurons by repeated creation of ephemeral excitatory processes, then there must be in the nervous system mechanisms capable of producing bombardment of the neurons at high frequency with volleys of impulses of subliminal density, *i.e.* with volleys that activate, on any one neuron, synapses, which being scattered all over the soma, cannot produce threshold stimulation. A neuron stimulated in manner will actually have a low threshold, because a few additional impulses will be sufficient to cause total activation of discrete zones of the synaptic scale and therefore initiate a new impulse (cf. Fig. 1). The bombardment of the motoneurons is a result of the activity of the chains of interneurons (cf. 1938*d*). An open chain like *M* in Fig. 3, provided that the impulses are able to cross the successive internuncial synapses, will cause bombardment of the motor nucleus at high frequency. That such chains actually exist and are active under physiological conditions was demonstrated some time ago (L. de Nó, 1928, p. 160), but open chains can maintain internuncial bombardment for only short intervals of time.

Enduring bombardment obviously requires repetitive passage of impulses through the same interneuron; therefore, it requires the existence of closed chains such as *C* in Fig. 3. Their existence has been implicitly accepted by several authors, Forbes, Cobb and Cattell (1923), Bremer and Rylant (1926), and more specifically by Ranson and Hinsey (1930) and the present author (1932, 1933*b*, 1934). As the absolutely refractory period of the neuron is about of the same order of magnitude as the synaptic delay, a closed chain with only two neurons may theoretically maintain the circulation of impulses (1934); practically, however, summation of subnormality, or in this specific case, true fatigue, will prevent the circulation after passage of very few impulses. Of course, if the chain includes a considerable number of links, the frequency of activation of each neuron will be sufficiently low to permit a prolonged circulation of impulses. Direct proof of the circulation of impulses through closed chains is not as yet available, but it is hardly necessary, because for conclusive anatomical reasons enduring bombardment unavoidably requires circulation through closed chains; therefore, as soon as the existence of bombardment has been demonstrated, circulation must be postulated.

The circulation may be thought of as being the result of automatic activity, *i.e.* of being created within the chain itself, hence the terms "reverberation" (Forbes, Cobb and Cattell, 1923), "reverberating chains" (Ranson and Hinsey, 1930) and "closed self-reëxciting chains" (Lorente de Nó, 1933*b*). Automatic activity may be assumed for chains with a large number of links, but for shorter chains it is easier to believe (cf. *Idem.*, 1938*d*, p. 230) that circulation is not automatic, but is maintained by impulses arriving from the periphery, or from other parts of the nervous system which find themselves in a state of activity.

A possible mechanism is illustrated in Fig. 12. Let it be assumed that fiber f_2 is conducting a series of rhythmic impulses initiated, for example, in a peripheral end-organ such as the labyrinthine maculae. The f_2 impulses are able to stimulate neuron *2* above threshold, but they stimulate neurons *1* and *3* subliminally. If now fiber f_3 is made to conduct an impulse which happens to be synchronous with one of the f_2 impulses, neuron *3* will fire, with the result that the impulses fed back to neuron *3* through chain *C* will summate with successive f_2 impulses and cell *3* will henceforth be able to conduct every impulse arriving through fiber f_2. Circulation in chain *C* and transmission through cell *3* will stop when any of the links in the chain, owing to repeated activity, acquires a high threshold and fails to transmit the circulating impulses. If the rise in threshold is created by an extra impulse arriving through a fiber like *d*, then the process may be called active inhibition (*Idem.*, 1938*d*, p. 238; cf. Gasser, 1937*c*).

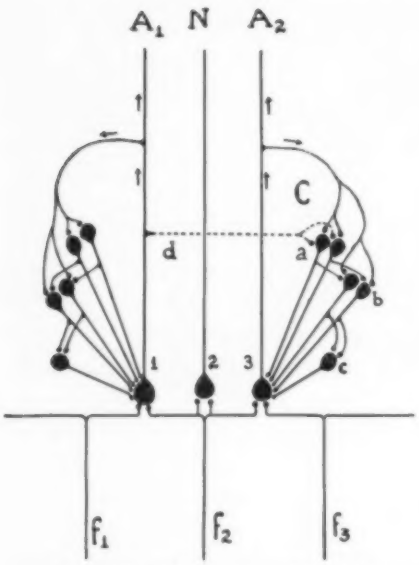

FIG. 12. Diagram explaining the production of reflex reversal by concurrent stimulation of two fibers (fibers f_1 and f_2 or f_2 and f_3) from different peripheral sense organs and its maintenance by the impulses conducted by the closed chain *C*, after fiber f_3, which initiated the response of cell *3*, ceases conducting. Maintenance of response through *3*, in the absence of impulses conducted by fiber f_3, would be called central after discharge. After discharge would cease as soon as the neurons in the closed chain *C* should acquire subnormal threshold. But if the subnormal threshold should be created by an extra discharge of cells of chain *C* caused by impulses conducted by collateral *d*, the phenomenon would be called active inhibition. Each one of the links in the closed chain represents a multiple chain of neurons such as chain *M* in Fig. 3 (from Lorente de Nó, 1938*d*, Fig. 12).

Indirect evidence of internuncial activity. By recording the responses of the motoneurons after conduction through the motor nerve, only indirect proofs of the activity of the internuncial system can be obtained. There is no need of presenting the available evidence again in this report, since it has been recently described (Lorente de Nó, 1936, 1938*b*, *c*, *d*). Only one point will be emphasized here.

Modification of the activity of the internuncial mechanism readily explains all the characteristics of the facilitation curves (Fig. 8, 9, 13). The variability of the facilitation curves observed in different preparations, or even in the same preparation under different conditions, finds a plausible explanation in the fact (*Idem.*, 1928, 1933*b*) that changes in the activity of the chains of interneurons may result in most varied alterations of the response to a given stimulus. This fact is indeed not surprising when it is realized that

synaptic transmission through any chain of neurons is optional (Fig. 2) and that the internuncial system includes many thousands of neurons (cf. *Idem.*, 1938*d*, Fig. 1); it certainly has many hundred times more neurons than the motor nuclei.

Thus, there is no difficulty in explaining why, although facilitation usually begins 0.5–0.6 msec. after delivery of the conditioning shock, it may sometimes start earlier, at 0.4–0.5 msec., and at other times much later, at 1–2 msec. The response to the testing shock becomes larger when the testing

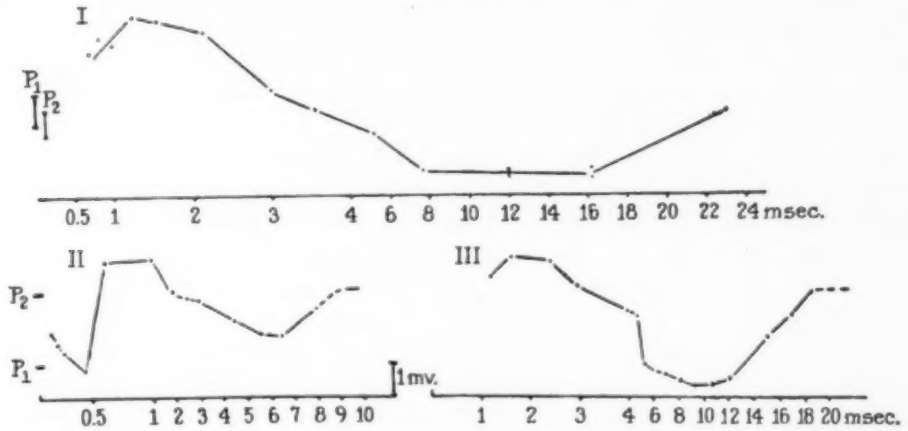

FIG. 13. Oculomotor preparation; responses recorded from the internal rectus muscle. Stimulating electrodes in position F_1 (Fig. 3). The curves are plots of the height of the testing response against the interval between conditioning and testing F shocks.

1. (Expt. 25-VI-34). Conditioning shock 120/100 stronger than the testing shock. The heights of the conditioning and unconditioned testing responses were not constant, but varied between the limits indicated at the left of the curve, P_1 and P_2. It is to be noted that after a short period of facilitation a period of inhibition (extinction) developed.

2. (Expt. 9-I-35). Conditioning shock 60/100 of the testing shock. P_1, height of the response to the conditioning shock; P_2, height of the response to the unconditioned testing shock. It is to be noted that after an initial period of depression ending at the interval of 0.5 msec. between shocks (cf. Fig. 9, *4*), a short period of facilitation appeared which was followed by a period of inhibition.

3. (Expt. 9-I-35). Conditioning shock 120/100 of the testing shock. The response to the conditioning shock had a height slightly larger than P_2 in curve *2*. The unconditioned testing response had the height P_2. The conditioning shock was followed by a period of unresponsiveness, attributable to refractoriness of the fibers that had been stimulated; afterwards there was a short period of facilitation and then a period of inhibition (from Lorente de Nó, 1936, Fig. 1).

impulses meet in the motor nucleus a sufficiently dense volley of internuncial impulses with which to summate; a sufficiently dense internuncial volley in some cases is produced when the impulses initiated by the conditioning volley have crossed through one internuncial synapse; but in other cases the internuncial volleys are not dense enough until after two or three internuncial synapses have been crossed (cf. *Idem.*, 1938*b*, Fig. 1; 1938*d*, Fig. 3).

Similarly it may be explained why facilitation, although usually lasting

for 6–8 msec., sometimes lasts only 1–2 msec. (Fig. 8, *1*) and at other times considerably longer, even 80 msec. (Fig. 8, *6*). A convincing explanation can also be offered for the fact that in some preparations (Fig. 13) the phase of facilitation may be followed by a phase of depression of the testing response, which is comparable to the "extinction" described by Dusser de Barenne and McCulloch (1934, 1935) of the responses initiated in the cerebral cortex by electric shocks. Brief facilitation means that the internuncial activity was increased for a short period of time, while prolonged facilitation indicates that the internuncial activity was increased for a correspondingly long time. And the appearance of inhibition ("extinction") is explained by the fact that after a period of increased activity the internuncial chains temporarily cease conducting the impulses of the tonic excitatory background (Lorente de Nó, 1936).

Direct evidence of internuncial activity. Direct proof of the activity of the internuncial system would consist in a recording of the internuncial impulses when they are entering the motor nucleus. In an early report (*Idem.*, 1935*d*, Fig. 3, *8*, *9*) it was indicated that an electrode placed on the pathways which have synapses on the oculomotor nucleus could be used to detect the passage of internuncial impulses, and that delivery of a shock to the posterior longitudinal bundle resulted in the arrival at the level of the electrode of a first wave of negativity, attributable to the impulses directly created by the shock, and then of several other waves which were attributed to impulses that had crossed through internuncial synapses. Technical difficulties however, prevented a satisfactory analysis of the phenomenon.

A new attempt to record the internuncial impulses has been made after several investigators had reported encouraging results obtained with micro-electrodes thrust into the brain (cf. especially the results of Renshaw, Forbes and Drury, 1938). It seems that the new experiments,* to be mentioned presently have afforded a satisfactory proof of the internuncial activity, and in addition have revealed significant data concerning the electric signs of the activity of somas of neurons. The records in Fig. 14–17 measure differences in electric potential between a micro-electrode placed near the active elements and a distant electrode located on inactive tissue, for example, the thalamus or the skull.

Distribution of bioelectric currents in volume conductors

This system of recording yields results quite different from those ordinarily obtained when a nerve surrounded by a dielectric is mounted on electrodes because, under these conditions, and in so far as the recording electrodes are concerned, the nerve acts approximately as a linear conductor of the currents of action, but the micro-electrode thrust into the brain-stem records differences in potential created by the spread of bioelectric currents in a volume conductor.

The problem of distribution of electric currents in a volume conductor was presented in a form suitable for the interpretation of neurophysiological problems by Helmholtz

* Cf. comparable experiments of Dusser de Barenne and McCulloch (1939) and of Bishop and O'Leary (1936).

(1853) and by Hermann (1879).* It follows from the theory of potential functions that whenever a nerve cell or fiber surrounded by a conducting medium becomes active and differences of electric potential are established between parts of the active element, electric currents appear in the medium in such a way that at any point outside the active element the potential† satisfies Laplace's equation. No appreciable current will pass through a point distant from the active element, and therefore, when pitted against a distant electrode, a micro-electrode will record a positive potential when it is located near the source of current, and a negative potential when located near the sink, the recorded potential being the greater the nearer the electrode is to the electromotive surface. Thus, the sign of the recorded potential is meaningless unless the position of the micro-electrode is accurately determined by subsequent histological analysis of the brain or by some other procedure.

It is a consequence of the physical laws which determine the distribution of current in volume conductors that the form of the recorded potential wave sign of the conduction of the spike process of a nerve impulse will depend, among others, upon two conditions: (i) the position of the micro-electrode in relation to the active element, and (ii) the direction in which the impulse is travelling. An example will bring out clearly the type of records that may be expected. Assume a nerve with an end in the air and the rest of the nerve, including the other end, submerged in a conducting medium. An impulse is initiated in the end in air and its passage along the nerve is recorded by three micro-electrodes, each pitted against an electrode located at some distant point of the medium. The first micro-electrode (i) is placed on the nerve at the point of entrance into the medium; the second (ii) on the nerve, somewhere between the point of entrance into the medium and the submerged end; and the third (iii) at the submerged end.

For a medium with the conductivity of the brain tissue and for lengths of nerve such as exist in the oculomotor preparation it can be predicted from the theory, and easily proved by experiment, that each of the 3 micro-electrodes will detect the existence of currents, as long as the nerve impulse occupies any part of the nerve; but the form of the recorded potential wave will be different for each micro-electrode. At *electrode 1* the potential wave will be diphasic (negative, positive) because when the impulse reaches the medium the point of entrance of the nerve is the first to become a sink; the recorded potential will have a negative sign until that point becomes a source; and since during the spike process no further reversal of current takes place, the second phase of the wave will remain positive until the current of the spike process ceases. At *electrode 2* the recorded wave will be triphasic (positive, negative, positive), indicating that that point of the nerve successively acts as a source, a sink and a source. The relative size and duration of the three components of the triphasic wave will of course vary with the position of electrode 2 on the nerve. For example, the first positive component will be very brief if electrode 2 is near the point of entrance of the nerve into the medium, or relatively very long if it is placed near the end; but all three phases will appear at any point of the nerve except as points 1 and 3. At *electrode 3* the record will again have only two phases, a first positive phase while that point acts as a source, and a second negative phase while that point is drawing current from points of the nerve in which the recovery is more advanced; since the end of the nerve is the last point reached by the impulse, point 3 will act as a sink until the end of the spike process.

* A more recent, but not as rigorous presentation, may be found in Wilson, McLeod and Barker (1933); interesting model experiments have been described by Bishop (1937); cf. too, O'Leary and Bishop (1939).

† The word potential is used strictly in the sense defined in mathematical physics. "Potentials" means values of a potential function, that in the discussion in the text is assumed to owe its existence to the presence of an electric current between parts of the active element (Fig. 25, II). The discussion made in the text would, therefore, not directly apply to differences of potential due to other kinds of processes. For example, it could not be directly applied to potentials generated by extracellular concentration gradients. In the text it is assumed, because it is permissible to do so, that at least during the short interval of time after stimulation that is considered in this report, the recorded potentials are preponderantly due to changes in the electromotive force of the polarized membranes of the somas and the axons.

Potentials recorded from the oculomotor nucleus and nerve

As in the case of the oculomotor nerve the length of the central segment of the nerve is short, the duration of the potential wave recorded from any point of the central segment of the nerve, or from the motor nucleus, should have approximately the duration of the spike potential as recorded from

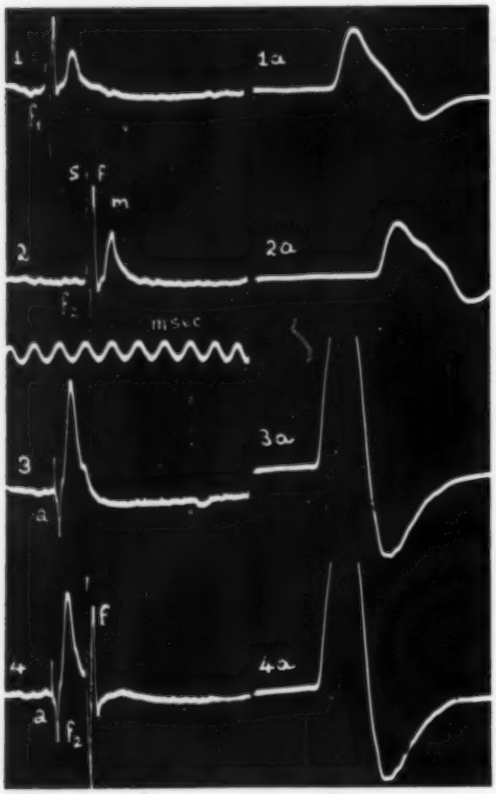

FIG. 14. Oculomotor preparation; responses recorded with two oscillograghs, from the motor nucleus (*1* to *4*) and from the internal rectus muscle (*1a* to *4a*). (Expt. 16-IV-39). Stimulating electrodes in position *F1* (Fig. 3). The responses from the motor nucleus were recorded with a micro-electrode about 50μ in diameter introduced into the nucleus at the point where the motor axons collect in a dense bundle to form the motor nerve. The indifferent electrode was placed in the oral and dorsal portion of the thalamus, *i.e.*, in a part of the brain that is not reached by the *f* impulses.

1 and *2*. Potentials developed by *F* shocks. *s*, shock artefact; *f*, potential of the *F* impulses; *m*, potential of the motoneurons; *1a* and *2a*, the responses of the internal rectus muscle.

3. Potential developed by the entrance of a maximal antidromic volley *a* into the motor nucleus. *3a*, response of the internal rectus muscle.

4. The *a* and f_2 shocks are delivered in rapid succession. The antidromic volley, by creating refractoriness in the motoneurons, obliterates the largest portion of the *m* wave. *4a*, response of the internal rectus muscle. The negative deflection in *3* measures well over 6 millivolts. Time in msec. between records *2* and *3*.

nerve with the ordinary technique, plus the conduction time along the nerve. Contrary to this expectation it has been found that a micro-electrode may record potentials of much longer duration. Therefore, in addition to the "travelling" process which gives rise to the ordinary spike potential, it must be assumed, that there exists some other "standing" process, which results in enduring differences of potential between different parts of the motoneuron, presumably between the soma and the initial segment of the axon. This significant result will be presented here in some detail.

Records *1* to *4* in Fig. 14 were obtained with the micro-electrode near the point where the motor axons collect into a dense fasciculus to leave the nucleus. Thus, in so far as the motoneurons are concerned, the records may be said to indicate the existence of currents that are leaving or entering the initial segment of the motor axons. In the position in which it

was placed, the micro-electrode also was sufficiently near the posterior longitudinal bundle to record differences of potential created by the passage of impulses through this bundle. A shock was delivered through electrodes *F* (Fig. 3, *F1*) with the result that the posterior longitudinal bundle conducted a volley of impulses (*f*) which resulted in discharges of motoneurons (Fig. 14, *1a*, *2a*). The micro-electrode recorded besides a sharp shock artefact (*s*, Fig. 14, *2*), the impulses conducted by the posterior longitudinal bundle (*f*) and then the motor impulses (*m*).

The *f* potential wave should have shown three phases, because the *f* impulses are conducted past the recording micro-electrode. The two first phases (positive, negative) are clearly visible, but the third phase is obliterated chiefly by the potential wave caused by the motor impulses *m*.

That the *m* wave was due to the discharge of motoneurons is easily proved. Delivery of a maximal antidromic shock (Fig. 14, *3a*) to the oculomotor nerve outside the brain stem resulted in a similar wave having of course a large initial phase of positivity. When shortly afterwards the *F* shock was delivered (Fig. 14, *4*), the *m* wave (Fig. 14, *2*) failed to appear, owing to the fact that the motoneurons were just beginning to recover from absolute refractoriness.*

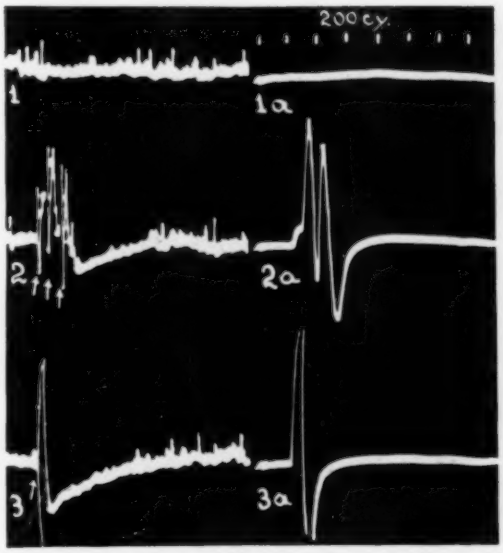

FIG. 15. From the same experiment of Fig. 14; but the amplification for records *1*, *2* and *3* higher than in Fig. 14.

1. Discharge of impulses of the tonic labyrinthine innervation, which, owing to the low amplification used, is not visible in record *1a*.

2. Three *F* shocks in succession, indicated by arrows, like f_1 and f_2 in Fig. 14, set up a synaptic response of motoneurons; *2a*, responses of the internal rectus muscle.

3. A maximal antidromic shock creates a volley of impulses entering into the motoneurons. Note that in *2* as well as in *3* the refractoriness of the motoneurons produces a silent period of the tonic discharge. Time, 200 cycles above record *1a*.

The records in Fig. 15 were obtained at higher amplification and reveal significant facts not clearly shown by the records in Fig. 14. Record *1* in Fig. 15, obtained without stimulation, shows a succession of small irregular spikes which obviously belong to those motor impulses that were maintaining the labyrinthine tonus of the eye muscles.

* It will be noted by comparing records *3a* and *4a* in Fig. 14 that a few motoneurons did respond, thus preventing satisfactory analysis of the small negative deflection which in record *4* follows the sharp negative deflection of *f*. This negative deflection in part must be attributed to the entrance of the *f* impulses in presynaptic fibers and synaptic knobs.

Record *2* was obtained by delivery of three approximately equal *F* shocks at intervals at which facilitation is usually observed. It will be noted in record *2a* that although the response to the first shock was small, the responses to the second and third were large; indeed, since all three responses make out a potential larger than that obtained after delivery of a maximal antidromic shock, there can be no doubt that all the motoneurons had fired and that at least the third response in *2a* included motoneurons that had already taken part in one of the previous responses.

Finally record *3* was obtained by delivery of a maximal (antidromic) shock to the motor nerve. Comparison of records *2* and *3* shows that the micro-electrode, besides recording sharp negative deflections, which approximately signal the passage of the *travelling* spike process, also recorded an enduring positive deflection, which undoubtedly indicates the existence of a *standing* difference of potential. As in records *2* and *3* all the motoneurons had responded, the final positive phase is approximately equal in both cases, the slight difference being attributable to the fact that in the case of record *2* the motoneurons fired in successive volleys.

The interpretation of the prolonged positive phase is not an easy matter. As already pointed out, the fact that the micro-electrode recorded a change of potential in the positive direction indicates only that it was located near a source of current and that some other part of the active element was a sink for that current. That during the third phase of the recorded wave the initial segments of the motor axons are sources of current is beyond doubt, but concerning the position of the sinks the evidence is not so conclusive. When the micro-electrode is moved peripherally along the central traject of the motor nerve, the third phase in question rapidly diminishes in size, but without reversing its polarity. This fact indicates that the main sinks are not located in the more peripheral parts of the axon. Therefore, they must be located in the soma of the motoneurons, and in fact, in the case of the hypoglossus nucleus, in experiments conducted especially for that purpose, the potential differences are by far greatest within the motor nucleus and in the zone where the axons collect to leave the nucleus. Furthermore, records have been obtained with the micro-electrode placed in the nucleus in which the potential wave, initiated by the entrance of antidromically conducted impulses, had only a first positive and a second negative wave, which ended at about the same time as the third or positive phase of the wave recorded from the initial part of the axons. However, the conditions for recording potentials are often unfavorable, because in the act of introducing the rather coarse micro-electrode (about 50μ in diameter) into the motor nucleus, the neighboring motoneurons are compressed and finally killed (cf. O'Leary and Bishop, 1939) before the micro-electrode is sufficiently near to any of them to be affected by the currents, that are entering the soma of motoneurons in a much higher degree than by the currents which are leaving neighboring axons. Thus, the conclusion derived from the experiments in question is

not definitive. It may be stated in the following sentences. The evidence available at present indicates that after conduction of an impulse initiated by synaptic or antidromic stimulation, the soma of the motoneurons remains negative in relation to the initial part of the axon, *i.e.* draws current from the latter during considerable periods of time, according to records such as those in Fig. 15 for 25–30 msec., or even longer. A later reversal of current has not been observed, but since the amplification was never high, a late reversal might have been overlooked.

Apparently, then, the temporal course of the depolarization and repolarization taking place during activity is not the same for the soma as for the axon of the motoneuron, as the depolarization in the former lasts for a longer period of time than the depolarization of the axon during the familiar spike process.

Comparison of potential differences in motoneuron with nerve potentials. An important peculiarity of the standing potential difference at the motoneuron *i.e.* of the relative negativity of the soma, is that it is a sign of depressed synaptic excitability. It will be noted in Fig. 15, *2* and *3* that during the third phase of the potential wave the tonic discharge of motor impulses visible in Fig. 15, *1* was first completely stopped and then progressively reëstablished as the potential difference diminished. This phenomenon is analogous to the familiar "silent periods" in reflex discharges (Hoffmann, 1920) which at present are generally attributed to subnormal excitability of the elements involved (cf. Gasser, 1937*a*, p. 200). Furthermore, it will be noted that the recovery curve of the motoneurons (Fig. 6, *2*) parallels in a striking manner the third phase of the potential wave in Fig. 15, *2* and *3*.*

In this respect the enduring negativity of the motoneurons is not directly comparable to the negative after potential of nerve, because during the latter the excitability of the nerve is supernormal (cf. Gasser, 1937*a*, p. 174). There is still another reason that prevents a direct analogy. The after potentials indicate the existence of differences of electric potential between points of the nerve which have been traversed by the impulse and a killed zone of the nerve which has not conducted an all-or-nothing disturbance; but in the case of the motoneuron the recorded differences of potential are established between two parts of the element that have conducted the same impulse. That after conduction the active soma of the motoneuron becomes negative

* The electrical signs of the activity of the oculomotor neurons reported here are not directly comparable with the electrical signs of the activity of spinal motoneurons described by Eccles and Pritchard (1937) and Eccles (1939) and also mentioned by Gasser (in this Symposium). The differences in the recorded potentials, however, might be attributable to differences in the recording techniques that have been used. On the other hand, the recovery cycle of ocular motoneurons (Fig. 6, *2*) appears to be different from that reported for spinal motoneurons. The differences are great when comparison is made on the basis of the description of Eccles and Pritchard, but become insignificant when the comparison is based on the observations of Gasser. As the present author has had no experience with spinal motoneurons, detailed discussion of these points would be unprofitable.

in relation to the active axon does not indicate what either of them would show when pitted against a part of the axon which by suitable injury has been deprived of the ability to conduct impulses. It appears, therefore, that in the present state of knowledge it would be premature to attempt a close comparison of the enduring phase of relative negativity of the soma of the motoneuron with potentials measured in nerve by means of ordinary techniques (cf. the more detailed discussion at the end of this report).

Potentials recorded during internuncial activity. Electrical signs of inter-

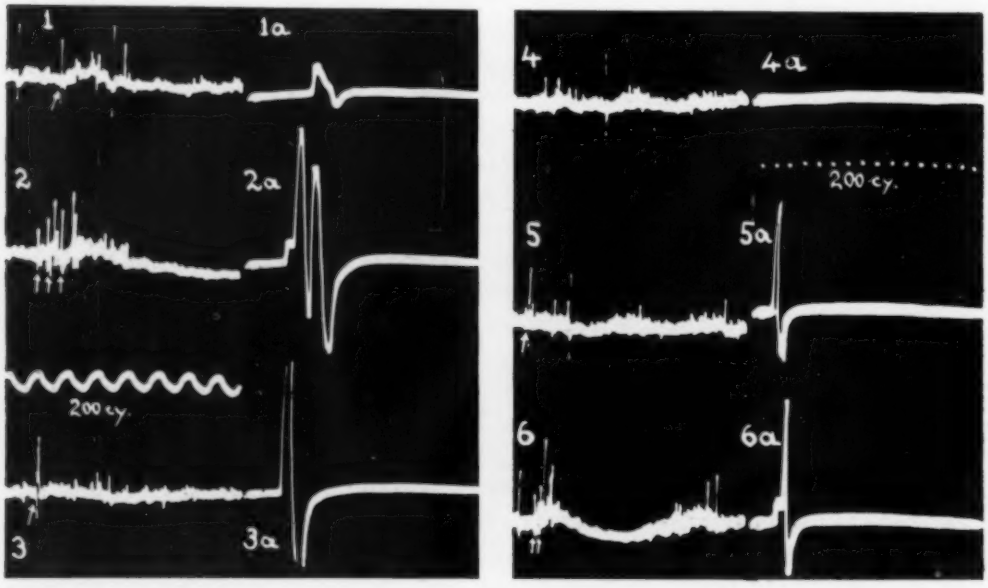

FIG. 16. From the same experiment of Figs. 14 and 15. *1* to *6*, responses recorded from the internuncial nuclei with a microelectrode introduced at about the center of the nucleus labelled *M.b.* in Fig. 3.

1. One *F* shock, marked by the arrow, sets up a moderate increase of the internuncial discharge. *1a*, response of the motoneurons recorded from the internal rectus muscle.

2. Three *F* shocks, marked by the arrows, like those used in Fig. 15, *2*, set up a marked increase of the internuncial activity. *2a*, response of the motoneurons.

3. A maximal antidromic shock, marked by the arrow, does not alter the internuncial activity. *3a*, response of the internal rectus muscle. Time for records *1* to *3*, 200 cycles between records *2* and *3*.

4. No stimulation. The sharp spikes belong to impulses of the tonic internuncial discharge.

5. A maximal antidromic shock, marked by the arrow, fails to alter the internuncial activity.

6. Two *F* shocks, marked by arrows, cause an increase of the internuncial discharge, followed by a silent period; the discharge is reëstablished when the internuncial elements recover from refractoriness. Time, 200 cycles between records *4a* and *5a*.

nuncial activity can easily be recorded; nothing else is needed but to introduce the micro-electrode into a pool of interneurons. For example, the records in Figs. 16 and 17 were obtained with the tip of the micro-electrode in-

troduced into a pool of interneurons, oral, lateral and ventral, to the oculomotor nucleus, *i.e.* about at the center of the pool labelled *M.b.* in Fig. 3 (cf. 1938*d*, Fig. 1. *H*).

Records obtained at low sweep speed (*4*) in the absence of stimulation show signs of persistent activity, because they contain an irregular series of spikes that signal the passage of impulses which are either arriving at or leaving the internuncial pool. The internuncial activity was of course not changed when maximal antidromic shocks were delivered to the oculomotor nerve.* There appeared (cf. the arrows in *3* and *5*, Fig. 16) a sharp triphasic

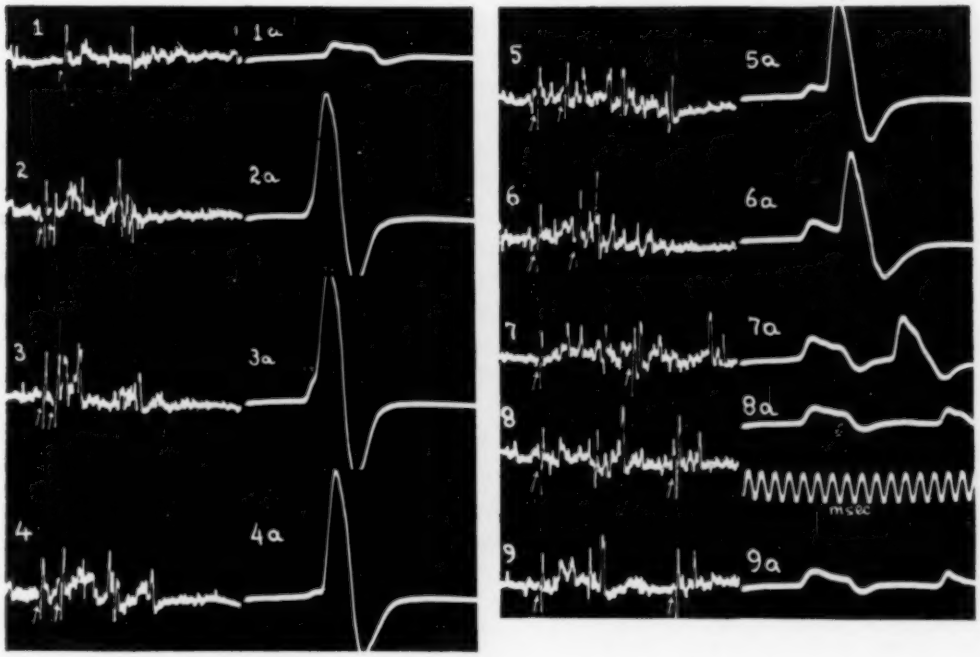

FIG. 17. From the same experiment of Fig. 14–16. *1* to *9*, responses of the internuncial nucleus, as in Fig. 16. *1a* to *9a*, responses of the internal rectus muscle. Time, 1000 cycles between records *8a* and *9a*. The records illustrate the relation between internuncial activity and facilitation of the motoneurons. Further details in text.

spike, a sign that the motor impulses on their way to the motor nucleus had passed at some distance from the micro-electrode, but the internuncial activity was not altered.

In contrast, an *F* shock to the posterior longitudinal bundle, which resulted in a small response of the motor nucleus (Fig. 16, *1a*), caused an ap-

* Records such as *3* in Fig. 14 and *3* and *5* in Fig. 16 conclusively prove that conduction across the synapses on the motoneurons is irreversible. Synchronous activation of all the motoneurons failed to set up impulses in fibers having synapses on them. There is no doubt that if the synapses had transmitted in the antidromic direction, the new impulses would have been recorded.

preciable increase of the internuncial activity (Fig. 16, *1*). The increase was much more pronounced when three *F* shocks were delivered in succession. The motor response (Fig. 16, 2*a* was then as strong as in the case of Fig. 15, 2*a*, and, as had been predicted, there appeared immediately after the *F* shock a spectacular increase of the number of internuncial impulses passing near the micro-electrode (Fig. 16, *2*). A remarkable peculiarity of the phenomenon was that the temporary increase was followed by a marked, also temporary, decrease of the internuncial discharges. When recorded at slow sweep speed (Fig. 16, *6*) the decrease of activity following the initial increase again had the appearance of a "silent period" with a similar electrical sign and with only slightly longer duration than the silent period in Fig. 15, *2* and *3*. One would, therefore, be inclined to conclude that, following activity, the internuncial axons for a certain period of time also remain electropositive in relation to their somas.

The suspected close correlation between the changes of activity of the internuncial pools and the excitability of the motor nucleus was found. The period of facilitation of the *F* responses is well illustrated in Fig. 17. A testing shock created a mild increase of internuncial activity (*1*) and initiated a small motor response (*1a*); but when this shock was preceded by another smaller shock (cf. Fig. 14, f_1, f_2) both the size of the motor response (*2a*) and the degree of the internuncial activity (*2*) increased enormously. Upon examining the records in Fig. 17, it will be noted that when the testing shock was delivered during the period of increased internuncial discharge (*2–7*), the testing response was facilitated (*2a–7a*); when, however, the testing *F* shock was delivered after subsidence of the increase of internuncial activity (*8*, *9*), the testing response did not show any facilitation (*8a*), but rather a depression (*9a*). In this experiment slight spontaneous variations of excitability of the preparation (cf. the conditioning responses in records *4a*, *5a*, *6a*, *7a*) prevented an accurate study of extinction after a single conditioning shock, but in other experiments in which the observations could be properly made, it was established that during the "silent period" of internuncial activity (Fig. 16, *6*) there was an inhibition (extinction) of the testing response exactly as it had been concluded (1936) from evidence not so direct.

It is an immediate consequence of the anatomy of the internuncial system, and therefore an incontrovertible fact, that at least a large number of the internuncial impulses recorded in Fig. 16 and 17 entered the motor nucleus and were delivered to the motoneurons. Therefore, the experiment illustrated in Fig. 16 and 17 constitutes final proof that a shock to the posterior longitudinal bundle initiates a volley of impulses, which not only is delivered to motoneurons and eventually causes them to respond, but also to interneurons and stimulates a number of them to discharge new impulses; this internuncial discharge increases the already existing internuncial activity during a certain period of time. While that activity is increased, the motoneurons are submitted to a powerful bombardment, which lowers their threshold and eventually may result in new motor discharges (cf.

1938*d*, Fig. 3). The increase of internuncial activity may subside gradually, in which case nothing but a decrease and final disappearance of facilitation are observed; but it may also happen that the increase in internuncial activity is followed by a "silent period" of the internuncial chains, in which case the facilitation of motoneurons is followed by inhibition.

III. General Interpretations*

In view of the evidence now available there can be no doubt that in a first approximation it is permissible to explain the activity of the nervous system in terms of the activity of chains of neurons and the detonator actions of the nerve impulses, *i.e.*, in terms of all the anatomical elements that the nervous system contains and of the nerve impulses that they produce or carry. Moreover, explanations of this type are forcibly demanded by a considerable body of established facts. When working out the theories in detail it may, and it probably will be found, that consideration of the activity of the chains of neurons, the properties of synaptic transmission and the changes in threshold during the recovery cycle of neurons which have been active—and these were the only factors considered in detail in a previous report (1938*d*)—is not sufficient to explain in a satisfactory manner all the known facts. Then, it will be necessary to introduce corrections into the theoretical arguments, taking into account the possible development of of a second phase of summation in subliminally stimulated neurons, chemical changes or changes of ionic concentration in the environment, possible influence of the action currents of active upon inactive elements (cf. Adrian, 1932; Jasper and Monnier, 1938; Eccles and O'Connor, 1938; Katz and Schmitt, 1939), and perhaps other as yet unknown or unsuspected processes (cf. 1938*d*, p. 240, footnote.) In certain cases the corrections may prove to be of paramount importance because the neuron works at threshold, *i.e.* it fires as soon as its threshold is reached, and the transmission through the chains of neurons is a conditional event; but still the basic assumption in the interpretation of the activity of the nervous system must be made in terms of the activity of the internuncial system. It is obvious that in the central nervous system internuncial bombardment is the primary factor which creates all the others. In its turn, however, the internuncial bombardment will be modified by the changes that it creates.

For example, it is known that under proper environmental conditions a nerve cell may become rhythmically active (cf. Bronk, in this Symposium). Let it be assumed as Barron and Matthews (1938) have done, that the environmental change required for initiating rhythmic activity of neurons takes place in the central nervous system during normal function. Then it would be possible to single out any interneuron and consider it a pacemaker, but as soon as this had been done, it would have to be realized that the pacemaker could not keep on driving other neurons by virtue of its own auto-

* Cf. the similar views held by Dusser de Barenne and McCulloch, 1939.

matism, because if the pacemaker forces other neurons to discharge, impulses will be delivered back to it through the internuncial chains, with the result that the driving pacemaker will then be driven by its fellow interneurons!

Theories of synaptic transmission

The experiments mentioned in this report have revealed data that have significant bearing on the problem of synaptic transmission of impulses to motoneurons. The information obtained defines the temporal course of transmission, establishes certain relations between the arrival of impulses at synapses and changes of electric and synaptic threshold of the neurons, etc. Thus it may be said that the experiments herein reported help to state the problem of synaptic transmission to motoneurons in precise terms, but they are insufficient to solve the problem.

Since it has been demonstrated that the soma of the motoneuron, whereupon the synapses are located, is electrically excitable (1935*f*; Barron and Matthews, 1938) and it may be taken for granted that the nerve impulse at its arrival at the synapses creates there differences of potential, it is permissible, even logical, to assume that the soma of the motoneuron is stimulated to discharge an impulse by these differences of potential. One might go so far as to believe that the available evidence is adequate proof of the essentially electrical nature of the process of transmission, but it must be realized that if the assumption were made—and such an assumption has indeed been made (Feldberg and Vartiainen, 1934)—that at the synapse a chemical agent is released in such a way that its production, action and inactivation follow a temporal course more or less comparable to that of the action current of the nerve impulse,—then, of course, the experimental facts would be compatible with chemical transmission (cf. 1935*f*, p. 69); and, if postulates such as those suggested by Barron and Matthews are made, then, the differences between chemical and electrical transmission may be reduced to almost pure formality (cf. Barron and Matthews, 1938, p. 315).

The difficulty consists in ascertaining whether the assumptions are supported by factual evidence, and, if this is the case, how far they may be developed; the difficulty is indeed not negligible, because it will happen that even the "facts" must be rechecked. For example, Feldberg and Vartiainen (1934) believed they had established beyond doubt that acetylcholine is specifically released at the ganglionic synapses during the act of transmission. Much was based on their conclusions and a finished theory was developed (cf. Dale, 1938), but upon reëxamination of the factual material the present author found (1938*a*) that acetylcholine is neither a specific product nor released with the regularity and temporal course that had been assumed.* The new findings are not entirely incompatible with chemical

* The validity of my experiments with perfusion of the superior cervical and nodosum ganglia (1938*a*) has been questioned by MacIntosh (1938–9). MacIntosh denies almost every statement that I made; but since Lissák (1939), in agreement with my results, re-

transmission, but if transmission by the synaptic release of acetylcholine should be maintained, important modifications would have to be introduced in the original theory. The need of revision is also shown by other circumstances, of which a few will be mentioned here. Chemical transmission with essentially different characteristics was postulated by Rosenblueth (1934) and strongly supported by Forbes (1934); but more recently Cannon and Rosenblueth (1937, and later papers) have formulated the chemical theory in a manner that deviates from the views formerly held by Rosenblueth (1934) and also from the views held by the London school. Brown (1937) and Eccles (1937) from their discussions of the same factual material, arrived at opposite points of view. Thus, Eccles concluded that acetylcholine is not the synaptic transmitter to ganglion cells, although it might play some rôle, among other things, on lowering their thresholds. This view, which is untenable on the basis of the postulates set forth by Dale (1938), is nevertheless compatible with the observations of the present author (1938*a*), with the experimental results of Bronk, Tower, Solandt and Larrabee (1938), and with facts presented by Bronk in this Symposium. Again, in order to assume that acetylcholine could be a facilitative agent, assumptions would have to be made regarding the inactivation of acetylcholine which would be accepted by some investigators and rejected by others.

No valuable purpose would be served by making further quotations from the extensive literature that has accumulated in recent years. A number of conflicting hypotheses have been made and others will undoubtedly be made. While the discussions go on, the following statement seems reasonable that *the action currents of nerve impulses arriving at the synapses may prove not to be the agents for synaptic transmission, but everything happens as if they were.* How this sentence should be understood will appear in the discussion that follows.

A model of synaptic transmission

In the study of problems not easily accessible to direct experimentation it is often useful to examine the phenomena, which under similar conditions, appear in artificial models. In neurophysiology there is scarcely any need of justifying the use of models because the well-known core-conductor model of Hermann, the membrane model of Labes and the iron wire model of Lillie have played important rôles in advancing and diffusing knowledge. Models, it is true, do not solve problems, but they clarify concepts and suggest working hypotheses. For these reasons attention is now directed to a model of synaptic transmission.

One of the most important contributions ever made to neurophysiology was the publication by Wedensky (1903) of his detailed study of the properties of a block of conduction in nerve. Wedensky, after comparing the

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ports release of acetylcholine by the vagal ganglia and this confirms my conclusion that acetylcholine is not a specific synaptic product, I may perhaps hope that some others of my conclusions were also correct.

properties of the block with those of the normal and the curarized neuromuscular junction, reached the conclusion that the block imitated conditions found at the synaptic junction. Wedensky's block is the synaptic model that will be studied here.

Observations of Wedensky. Wedensky and his co-workers used a large number of blocking agents (cocaine, phenol, heat, pressure, galvanic polarization, intense faradization, etc.). While differences were found to exist between the various blocks,* properties were established common to practically all the blocks. For the purpose of the following analysis of the block, the three most relevant properties are: (i) A partial block can transmit impulses at low frequency, but fails to transmit impulses at high frequency. This phenomenon is usually known under the name of Wedensky inhibition. (ii) After the blocking agent is applied to the nerve, the threshold of the treated segment is found to rise progressively, while the threshold of the segment below the block becomes lower than it was before. Entrance of impulses into the treated zone produces in it an additional enduring rise in threshold. (iii) A block that has become complete, either because the blocking agent has acted during a sufficiently long time or because impulses entering into it have strengthened the previous partial block, fails to conduct impulses; but while the impulses are stopped at the upper margin of the block, the threshold of the nerve below the block is lowered, the lowering of threshold being a cumulative and enduring process (cf. Wedensky, 1903, p. 65). This phenomenon will henceforth be called "Wedensky facilitation" or "facilitation across the block."

From the extensive literature dealing with Wedensky facilitation, inhibition and concomitant phenomena, only a few papers will be mentioned here. First are the contributions of Samojloff (1925) and Woronzow (1924, 1931) who, with string galvanometer, analyzed in greater detail and with greater precision than Wedensky (cf. Wedensky, 1903, p. 92) the electrical signs of the modifications that take place at the block; and then mention will be made of two papers by Hodgkin (1937*a*, *b*) that brought forth an important advance in knowledge.

Temporal course of the facilitation across the block; initial phase. Hodgkin established the relationship between the temporal course of the electrical sign of the blocked impulse and the temporal course of facilitation. One recording electrode at or shortly below the block, when pitted against another electrode also below, but at considerable distance from the block, records an electronegative variation when the facilitating impulse is stopped at the block. The recorded difference of potential is one that might be expected from electrotonic conduction through the block of the electric sign of the blocked impulse. The important fact is that the lowering of threshold at points below the block quantitatively follows the course of the recorded potential difference. In Hodgkin's observations the potential recorded below

* A remarkable study of blocking agents has recently been made by Bishop (1934).

the block usually included a sharp spike-like deflection only, but in some cases it also showed a tail, which Hodgkin interpreted as being attributable to the negative after-potential of the blocked impulse.

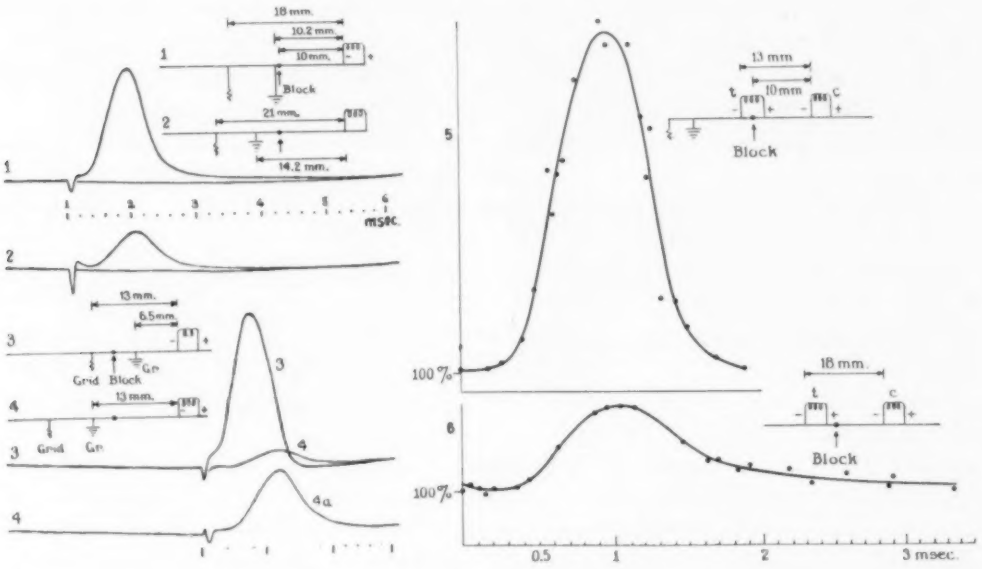

FIG. 18. Facilitation across a Wedensky block in the sciatic nerve of a green frog (Expt. 4-VIII-37). The block was created by applying to the nerve a cotton thread moistened with 0.75 per cent cocaine hydrochloride, at the point marked with *Block* on the nerve. Distances are measured from the cathode of the conditioning coil.

On the left side potentials recorded with the ground and grid electrodes placed at the points of the nerve, indicated in the diagrams with the same numeral as the records, after delivery of a maximal *A* shock through the conditioning electrodes.

1 and *2*. Potentials recorded below the block, the same amplification for both records; note the decrement and change of shape suffered by the spike-like potential (detonator negativity) during its transmission through four mm. of nerve, and note also that the detonator negativity is followed by a residual negativity relatively larger in *2* than in *1*.

3. The spike recorded above the block at an amplification lower than for records *1* and *2*.

4. The spike-like potential recorded below the block at the same amplification as for *3*. *4a*, the potential of *4* at higher amplification. On the right side, facilitation of the response to a submaximal shock produced by an impulse that is stopped at the block. Conditioning and testing electrodes in the positions indicated in the records. Abscissae, intervals between shocks in msec. Ordinates, height of the conditioned response; 100 per cent, height of the unconditioned response. Note that the ordinates scale in the upper curve is smaller than that of the lower curve. Compare shape of the facilitation curves with the potentials on the left side and note that in the lower curve the potential produced by surge to ground (shock artefact) also lowered the threshold of the nerve, apparently in a similar manner as the later arriving spike-like potential.

There is not much that can be added to the description of Hodgkin (1937*a*, *b*) of the initial phase of the phenomenon, except perhaps to present records (Fig. 21) which show in greater detail than those of Hodgkin the modifications suffered by the potential wave while it is being transmitted electrotonically through the block and points below the block. Also, it is

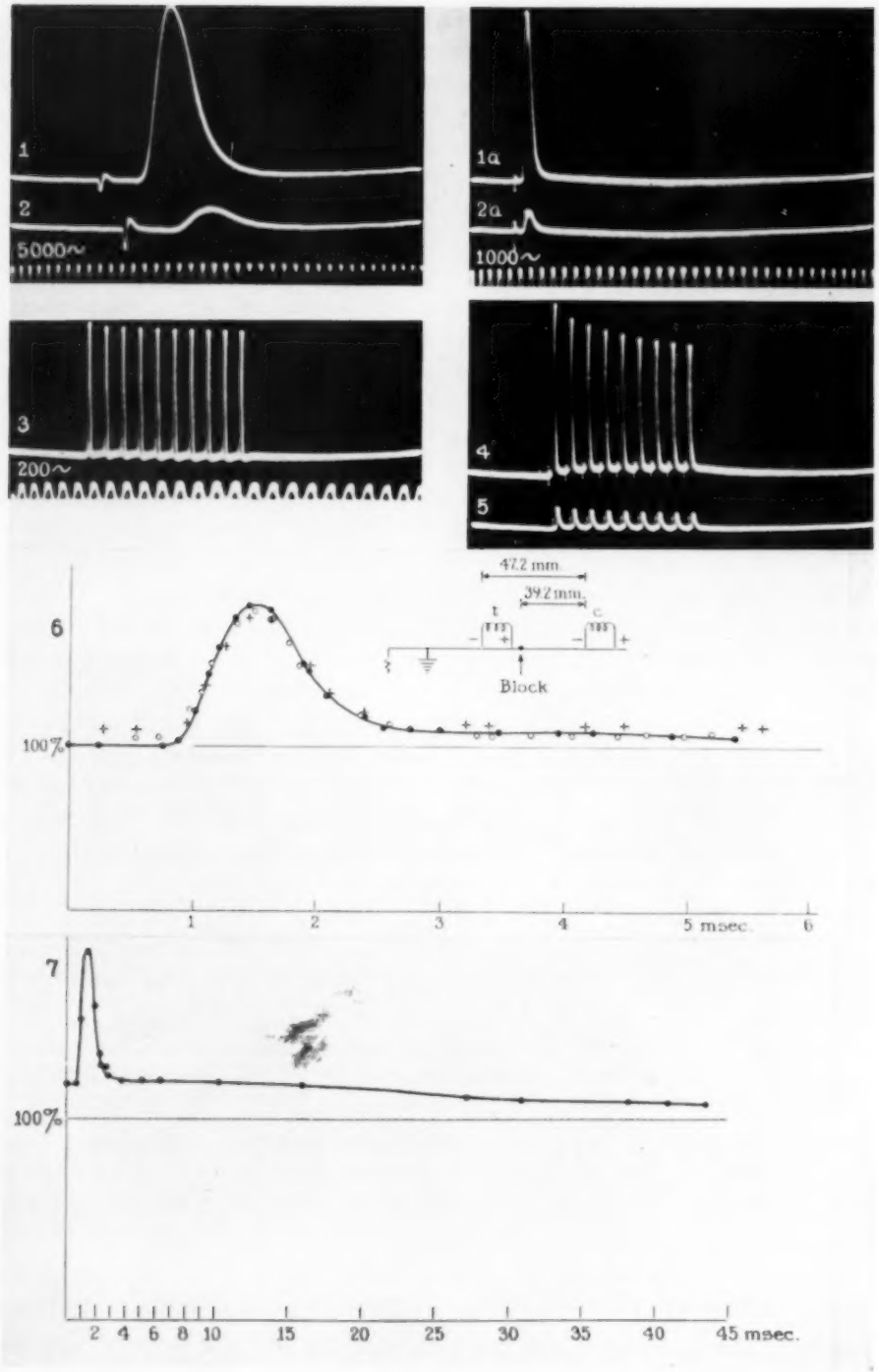

FIG. 19. (See next page for legend.)

pertinent to publish "facilitation" curves obtained with the testing cathode at different distance below the block (Fig. 18). After one impulse the electrical signs and the changes in threshold below the block, following the short interval of time considered by Hodgkin, are not great (Fig. 19, *6*), but when the block is conditioned by a train of impulses, the late phases of the phenomenon, which obviously played a significant rôle in Wedensky's experiments are very prominent (Fig. 19, *7*, and 20).

Facilitation by a train of impulses. Disregarding the existence of a demarcation current through the block and neighboring points of the nerve, a general description of the phenomena may be made in a few sentences. Arrival at the block of a train of impulses creates in the block, besides rapid changes similar to those observed after single impulses, a certain progressive change which, during and after arrival of the train, results in the creation of differences of electric potential between the block and points above and below it. When the currents are recorded with an electrode at or below, but near the block, and another electrode far below the block, the currents reveal themselves as a sequence of potentials resembling the familiar spike negative after-potential positive after-potential sequence. Negativity below the block indicates the existence of a lowered threshold, and positivity, the existence of a raised threshold, in comparison with the threshold before the conditioning. In this respect the relationship between excitability and electrical signs follows the rule that is given by Gasser (1937*a*, *b*; cf. also, in this Symposium) for true after-potentials, *i.e.* for the after-potentials recorded with a nerve that has conducted all-or-nothing disturbances. The absolute value, temporal course, and relative size of the three components of the potential sequence recorded below the block change progressively when the upper electrode is approached to the fixed electrode far below the block.

FIG. 19. Facilitation across the block produced by a train of impulses (Expt. 6-VIII-37). Bullfrog sciatic, cocaine block at 39.2 mm. from the conditioning cathode. Testing electrodes as indicated in the diagram, (*t*). Recording electrodes for the impulses initiated at the testing cathode also indicated in the diagram.

1. Potential recorded at the testing anode, used as recording ground and pitted against the recording grid, after delivery of a maximal *A* shock through the condition electrodes (*c*). *1a.* The same at a slow time line.

2. and *2a.* Potentials recorded at the testing anode at the same amplification as for records *1* and *1a*.

3. A train of ten impulses recorded with the ground electrode above the block, 10 mm. from the conditioning cathode. Note that all the spikes are approximately equally high. Much lower amplification than for records *1* and *2*.

4. and *5.* The train of impulses recorded at and below the block, under the same conditions as for records *1* and *2*. Note the residual negativity and the drop in height of the spike-like potentials.

6. Facilitation curves, ● after one impulse, ○ after two impulses, ×, after five impulses of the train reproduced in *3*, *4*, *5*. Note the residual facilitation that corresponds to the residual negativity.

7. Facilitation after ten impulses. Note the long lasting effect of the residual negativity.

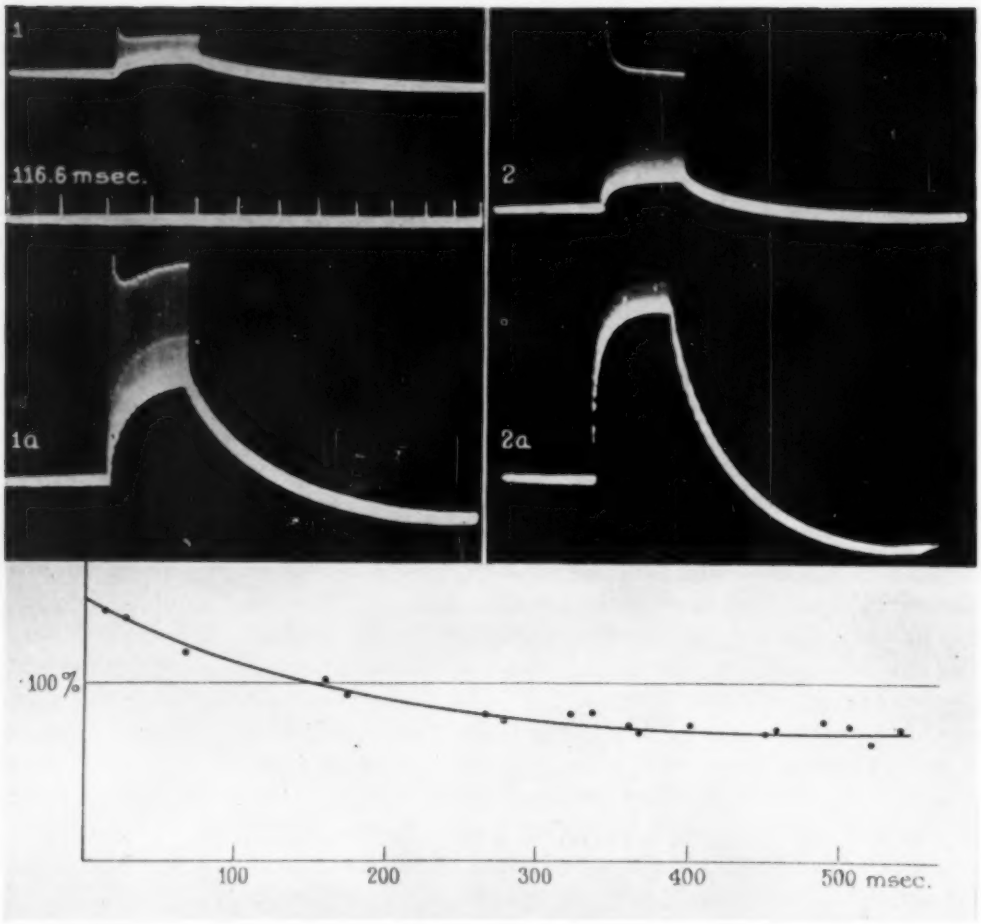

FIG. 20. Facilitation across a cocaine block. Bullfrog sciatic (Expt. 26-X-37). Conditioning by a train of impulses produced by shocks third of maximal *A* strength at the rate of 225 per second. When the train was recorded 11 mm. above the center of the block the spikes kept constant height, but when recorded at 6 mm. above the block they progressively diminished in height, thus indicating that cocaine had spread up to that point. Testing anode 2.5 mm. above center of the block (cf. Fig. 21, *Block*). Testing cathode 1.5 mm. below center of block.

1. Potentials recorded at the testing cathode connected as ground electrode and pitted against a grid electrode 29 mm. below the center of the block.

2. Potentials recorded at the testing anode. Same amplification as for *1.*

1a and *2a.* The potentials of *1* and *2* recorded at five and a half times higher amplification. Note that in *1* the cumulative residual negativity is much larger in relation to the spike-like potential than in *2*, and note also that the residual negativity goes over to positivity in *2a* about 100 msec. earlier than in *1a*. Below, facilitation curve obtained after a train of impulses equal to those used for obtaining records *1* and *2*. During the period of residual negativity at the testing cathode (*1a*) the threshold of the nerve was lower than the resting threshold, but during the period of positivity the threshold was higher and, therefore, the response smaller than when unconditioned.

There is no need of describing in detail the facilitation curves in Fig. 18, 19 and 20; it is sufficient to mention that the conduction block was obtained with 0.5 to 0.75 per cent cocaine hydrochloride, and to emphasize the similarity between the temporal courses of the changes in threshold and the recorded potentials. But a study of the recorded potentials will be made in some detail in order to compare phenomena at the block with phenomena at the synapse.

Spike-like potentials below the block. In Fig. 21 only the spike-like potentials are included. There are in this figure two sets of records. Those

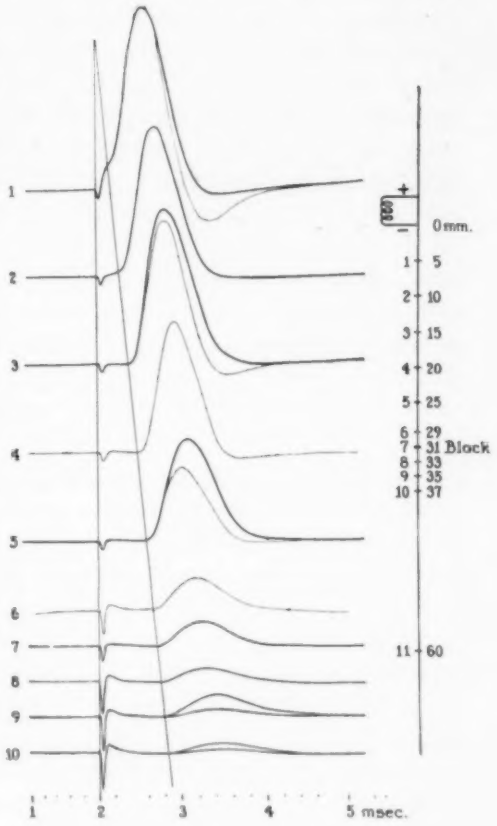

FIG. 21. Cocaine block, bullfrog sciatic (Expt. 26-X-37). Potentials recorded at different points of the nerve after delivery of a shock about one third of the maximal *A* strength. Position of the stimulating and recording electrodes indicated in the diagram at the right. The stimulating electrodes were kept at a fixed position as was the grid electrode, at point *11*, 60 mm. from the cathode, for the records traced with thick lines, and at point *7* for the records traced with thin lines. The recording ground electrode was successively placed at points *1* to *10* at the distances indicated in mm. on the diagram. The amplification was maintained constant, except that for records *9* and *10*, two different amplifications were used, the smaller being the same as for the other records. For record *6* the shock strength was inadvertently doubled, which produced a height of response about twice the height of the response that would have been obtained with the shock used for the other records. Note that the spike-like potential is decrementally transmitted through the block and below the block with the same apparent speed as the nerve impulse is conducted above the block. The initial deflection in record *1*, immediately before the spike is due to electrotonic conduction of the stimulating shock.

traced with thick lines were obtained with the fixed electrode on point *11* of the nerve, *i.e.* 29 mm. below the center of the block, while those traced with thin lines were obtained with the fixed electrode on point *7*, *i.e.* at the center of the blocked segment. Therefore, records *1*, *3*, *4*, *5* and *6* (thin lines) measure potential drops caused by currents at segments of the nerve above the block and at the upper margin of the block, while the other records (thick lines) include those potential drops algebraically summated with drops attributable to currents established between the block and points of the nerve below it.

An important fact is illustrated by records *1* to *6* (thin lines). Record *1* is diphasic, although the second phase is much smaller than the first one. The diphasicity is less marked in records *3* and *4*, and although very small, is still present in record *5;* but there is no sign of diphasicity in record *6*. Cocaine poisoning diminishes the conduction rate of the nerve impulse; therefore, owing to the lack of uniformity of the block, a progressive decrement of the rate of conduction took place when the impulse approached the block. This decrement, however, is not sufficient to explain the monophasicity of record *6*. When both recording electrodes are in contact with points of the nerve through which transmission takes place, the only case in which the recorded difference of potential does not cross the base line and change its sign is when the wave of negativity reaches the second electrode with sufficiently subnormal height (Hermann, 1878*a*, *b*). Where the decremental conduction began cannot be determined with accuracy because the spike included impulses in a number of fibers; but for the purpose of the present analysis, it is sufficient to establish the existence of the decrement at the margin of the block. As an important consequence of the decrement when the impulses reached the margin of the block, a steep potential gradient was created there—*e.g.* record *6* was obtained with the recording electrodes 2 mm. apart—with the remarkable peculiarity that the potential gradient had approximately the duration of the spike process as recorded at untreated points of the nerve and vanished without reversing its sign.

Detonator negativity at the synaptic knob

It is thinkable that a potential gradient similar to the spike-like potential gradient at the margin of the block is created at the synaptic knobs when impulses arrive there. It is true that if conduction through the knobs should take place in the same manner as in ordinary nerve fibers, the creation of a significant unidirectional potential gradient in the knobs, *i.e.*, in elements of microscopic size could hardly be assumed; but if the nerve impulse, when entering the fine branches of division of the presynaptic fibers and the synaptic knobs, should undergo a progressive modification comparable to the decrement observed at the margin of the block, then a steep potential gradient would appear at the knobs. This gradient would approximately have the duration of the spike process in the parent fiber and would vanish without reversing its polarity.

The spike-like potential gradient at the synaptic knob—henceforth to be called detonator negativity—would be responsible for the detonator action of the nerve impulse. Its duration would be that of the synaptic delay,—its maximum reached in a fraction of a millisecond,—and it would decay very rapidly so that effective summation of detonator action at neighboring knobs would be possible only when produced at very short intervals of time. In brief, a potential gradient of this kind would not only be a strong stimulus because it would force a dense current through the soma of the

neuron,* but it would also be a stimulus having all the known properties of the detonator action.

Response of the motoneurons to detonator negativity. It is to be expected that when the detonator negativity develops at the synaptic knob, a decremental wave of depolarization spreads over the neuron in a manner comparable to the spread of the spike-like negativity through and beyond the block that is shown by records *7* to *10* in Fig. 21. The depolarization must be greatest at the margin of the active knob and rapidly decrease with increasing distance. Except for the slight changes taking place during its propagation over the soma, the temporal course of the depolarization of the neuron should closely follow that of the detonator negativity at the synaptic knob and therefore, if the depolarization of the neuron has not been strong enough to initiate an all-or-nothing disturbance, it will disappear as fast as the detonator negativity itself.

Passive electrotonic transmission and local response in nerve. An important question that must now be considered, but cannot yet be answered, is whether the transmission across the block of the spike-like negativity is attributable to "passive" electrotonic propagation or, in addition, involves an "active" response of the blocked segment of the nerve below the block. Hodgkin (1937*a*, *b*) attributed the potentials recorded below the block to electrotonic transmission from the blocked impulse. This interpretation was based upon the fact that subliminal electrical shocks were found to propagate across the block in a manner similar to that of the potential of the blocked impulse. The question, however, must be reopened because recent work (Hodgkin, 1938; Arvanitaki, 1938, and earlier papers) has led to the conclusion that in crustacean nerves the all-or-nothing disturbance is preceded by a local potential, that is considered to be an "active" response of the nerve fiber. A "local" response at the cathode has been described for frog nerve by Katz (1937), although the evidence does not seem to be conclusive (Blair, 1938*b*). The conditions in multifibered nerves of vertebrates, however, are so much more complex than in crab nerves, that failure to demonstrate a local cathodal response may not be a conclusive proof that a local response does not develop before the all-or-nothing disturbance arises; but, on the other hand, it would be inappropriate to base theoretical arguments on the existence of a local potential in vertebrate nerve until some significant evidence of its existence has been found.

One type of evidence accessible to present-day techniques would be a demonstration of differences in the propagation of the electrotonus away from the cathode and away from the anode. It seems, however, that no apparent difference can be demonstrated.† Fig. 22 illustrates a pertinent experiment in which the propagation of electrotonus was measured in terms of the changes of excitability of the nerve. Curves *1*, *2*, *3* and *4* measure the changes of threshold at the testing cathode (*t*) after delivery of a subliminal conditioning shock through electrode *c*. For curves *1* and *2* the distance between the cathodes was two mm. and for curves *3* and *4*, seven mm. For curves *1* and *3* the conditioning shock was 91 per cent of the threshold and for curves *2* and *3*, only 30 per cent. Despite the fact that according to Katz' (1937) observations, the stronger shock should have produced a significant local response, the transmission of the cathodal effect was similar in both

* Since nerve fibers contain acetylcholine, there is no objection to the assumption that acetylcholine ions, as well as other ions, are involved in carrying the current; (cf. Barron and Matthews, 1938; and Eccles, 1936, p. 371). This movement of acetylcholine ions, however, would not result in a "release" of acetylcholine unless pathological conditions were created.

† For a discussion of the problem of the spread of electrotonus cf. Bogue and Rosenberg (1934) and for a similar problem that appears in the study of the conduction of the nerve impulse cf. Cremer, 1929, and Cole and Curtiss, 1939*a*, *b*.

cases, and it was also similar to the transmission of the anodal effect in the case of curves *5* and *6*, although anodal shocks are not supposed to create "local potentials."

It seems, therefore, advisable to leave this important question open, especially because, in addition to those mentioned by Katz (1937), there still are other differences between the cathodal and anodal curves (cf. 1938*e*) that perhaps could not be explained without the assumption of a specific response at the cathode.

Residual negativity

The processes at the block do not end after the rapid decay of the spike-like potential, because this is followed by an enduring negativity that mimics the negative after-potential of a true impulse (Fig. 18, *1, 2;* Fig. 19, *1a, 2a*). This residual negativity is greatly increased when the block is conditioned by a train of impulses. Record *6* in Fig. 23 shows that while the train is being stopped at the block, each impulse adds a certain amount to the residual negativity left by its predecessor, with the result that at the end of the train there still remains at the margin of the block a significant gradient with the same polarity as the gradient created by the spike-like process. Records *7* to *10* in Fig. 24 demonstrate that this gradient is also transmitted through the block to points below. On the other hand, an examination of records *1* to *6* in Fig. 23 proves that the gradient at the margin of the block is also transmitted in the opposite direction (towards point *1*). Since no records were taken from points between *4* and *7* (Fig. 24) it is not known at which point the maximal negativity would have been recorded; obviously however, it must

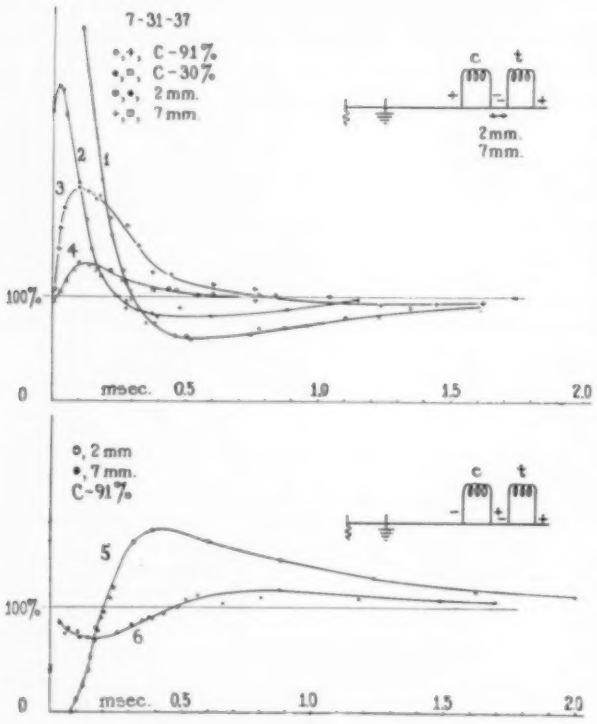

FIG. 22. Curves illustrating the transmission along the nerve of the effects of subliminal cathodal and anodal shocks. Green frog sciatic (Expt. 31-VII-37). The diagrams indicate the position of the conditioning (*c*) and testing (*t*) electrodes. In ordinates, height of the submaximal testing response conditioned by the subliminal conditioning shock at the intervals indicated in msec. in abscissae; unconditioned height indicated by the 100 per cent horizontal line.

1, 2, 3, 4. Cathodal curves. For *1* and *2*, cathodes at two mm. distance; for *3* and *4*, at seven mm. For *1* and *3*, conditioning shock 91 per cent of the threshold; for *2* and *4*, 30 per cent of the threshold.

5, 6. Anodal curves. Conditioning shock 91 per cent of threshold. For *5*, distance between conditioning anode and testing cathode, two mm., for *6*, seven mm.

have been maximal somewhere between points *4* and *6*. Therefore, it can be stated that in the whole nerve, immediately after the train of impulses, there was negativity in relation to point *11*, *i.e.*, to unchanged nerve, and that there was an increasing gradient of negativity from point *10* to points *6–4*, and from there on towards point *1* a decreasing gradient.

Similarity of potential at blocked segment with that at motoneurons after conduction of an impulse. A detailed consideration of the increasing gradient of residual negativity from points *10* to *6* (Fig. 21) will show that the block imitates a condition found in the motoneurons. As already mentioned, Wedensky demonstrated that when impulses enter a partial block they cause a rise in threshold in the block at the same time lowering the threshold below the block. Obviously, the demonstration of the rise of threshold at the upper margin of a complete block cannot be made with Wedensky's technique, but it can be obtained in oscillographic records. It will be noted in Fig. 19 that when the impulses are recorded above the block, the true spike potentials of all the impulses have the same height (Fig. 19, *3*); but when the spikes are recorded at or below the block, the spike-like potentials rapidly diminish in size (Fig. 19, *4*). This proves that the threshold at the margin of the block was raised by each successive impulse, so that the true conduction of each impulse ceased at a greater distance from the center of the block than the true conduction of its predecessor in the train, with the result that since the distance for decremental conduction was increased progressively, the size of the recorded spike-like potential decreased progressively. In records obtained at high sweep speed it was observed that the decrease in size was accompanied by a change in the shape of the potential

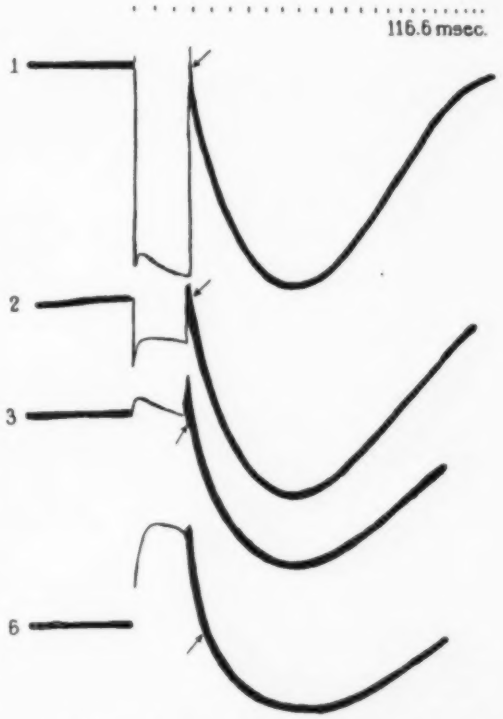

FIG. 23. Cocaine block. Same experimetn as in Fig. 20 and 21. Potentials recorded with the ground electrode successively at points 1, 2, 3 and 6 of the nerve, and the grid electrode at point 7 (Fig. 21). Stimulation by a train of shocks at the rate shown in Fig. 20. Amplification five times higher than in Fig. 21, and equal to the amplification in Fig. 20. The shock strength in Fig. 23 and 24, however, was three times higher than in Fig. 20. The tracings do not reproduce the spike-like potentials during the train, but indicate the lowest level reached by the spikes and therefore allow following the development of residual negativity. The arrows indicate the change of residual negativity into positivity.

and an apparent delay in the transmission that were like those observed in records *7* to *10* in Fig 21. In other words, the rise in threshold at the upper margin of the block produced the result that would be expected from displacing the recording electrode away from the block.

It is therefore clear that a partial block, after having been strengthened by transmission of one or several impulses, shows a distribution of potential (and also of thresholds) comparable to those observed during the phase of "standing" relative negativity of the motoneurons mentioned in previous paragraphs. (See Fig. 14 and 15.) In the block the greatest negativity is found at the upper margin which undergoes a rise of threshold, while the points below the block, which are electropositive in relation to the upper margin, undergo a lowering of threshold. Similarly, the soma of the motoneuron, after conduction of an impulse, becomes electronegative in relation to the axon and acquires a high threshold to synaptic stimulation (cf. Fig. 6, *2*) while the axon, despite its being relatively electropositive, has an excitability to induction shocks that is either very near normal or even supernormal (cf. Fig. 6, *1*). Obviously, there is no contradiction between this theoretical analogy and the fact that when tested with induction shocks, the excitability of the soma parallels that of the axon (Lorente de Nó and Graham, 1938). During the phase of "standing" electropositivity of the axon there must exist potential gradients in the soma and, therefore, it may be assumed that the current created by an induction shock may become effective at a zone of the soma comparable to points *7* to *10* (Fig. 21) of the nerve that is being taken as a model, while the synaptic stimuli may become effective at zones of the soma comparable to points *4* to *7* of the model.

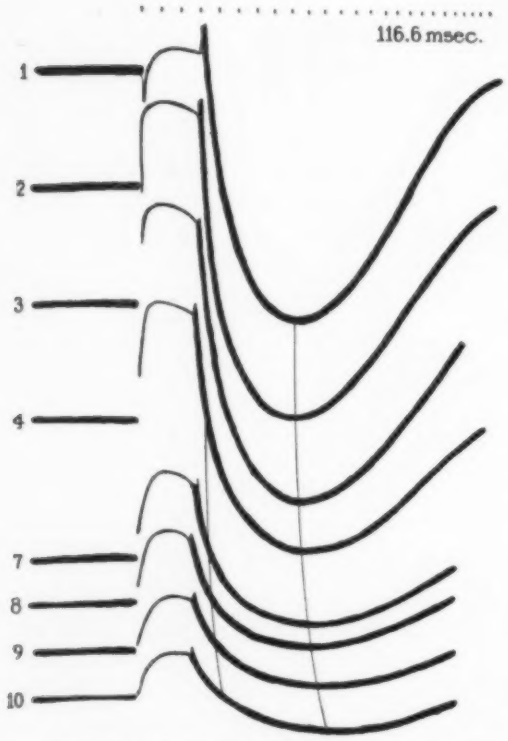

FIG. 24. Cocaine block. Same experiment as in Fig. 20, 21, 23. Same stimulus and amplification as in Fig. 23. The grid electrode was maintained at point *11* of the nerve (cf. Fig. 21), the ground electrode was placed at the points indicated on the left of the records. The thin lines through the records indicate when the negativity changed into positivity at the different points of the nerve, and when the positivity reached its maximum. Note that the maximum of positivity appeared later, the greater was the distance below the center of the block. Further details in text.

Transmission of residual negativity. The transmission of the residual negativity through and below the block can hardly have been a passive elec-

trotonic conduction. In comparing record *6* in Fig. 23 with records *7* to *10* in Fig. 24 (cf. Fig. 20, *1* and *2*), it will be noticed that the transmission of the residual negativity is much slower than that of the spike-like potential (Fig. 21), and that the progression of the wave, of residual negativity, is not maintained entirely by the gradient at the upper margin of the block, because when at point *7* no negativity is recorded, about half the maximal negativity is recorded at point *10* (Fig. 24). Furthermore, the residual negativity is transmitted with much less decrement than the spike-like process (cf. Fig. 20, *1* and *2*). Under conditions such as these the conclusion is unavoidable that transmission of the gradient of residual negativity was not "passively" electrotonic. It must have been accompanied by an "active" response of the points of the nerve through which it was transmitted. The nature of the response cannot be ascertained from its electrical sign, or, in other words, electrical records do not reveal the nature of the processes that accompany the transmission; but taking into account the long duration and slow transmission of the process it seems to assume that the cumulative negativity was primarily attributable to changes in the polarized membranes, comparable to those taking place during transmission of the spike and spike-like negativities, but also that the transmission of the residual negativity resulted in a significant displacement of those ions that maintain the polarization of resting nerve.

Residual negativity at synaptic knobs could cause second phase of summation. An enduring and cumulative gradient of residual negativity created at the synaptic knobs would explain the second phase of lowered threshold of ganglion cells stimulated by a subliminal volley of impulses (*c.e.s.* in Eccles' terminology). Since in the model under consideration the transmission of residual negativity across the block is accompanied by a certain response, in order to preserve consistency of thought, it must be assumed that the neuron produces a similar response when it is acted upon by residual negativities at the knobs. It should be expected that this response would spread over the neuron in a like manner as the residual negativity spread through points *7* to *10* of the blocked segment of the nerve (Fig. 24). Near the active knobs the effect of the detonator negativity would be many times greater than the effect of the residual negativity (Fig. 19), but the difference would decrease with the distance from the active knob (Fig. 20). For the case of stimulation by a rhythmic series of impulses, the effect of accumulated residual negativity at points distant from the active knob could finally become almost as great as the effect of the detonator negativity of the first impulse of the train (Fig. 20, *1*, *1a*). Therefore, the neuron would acquire a persistently lowered threshold and the possibility of a temporal summation of impulses arriving in succession through the same group of fibers would be created.

Also, if the train had a duration and frequency beyond physiological limits, the cumulative effects of residual negativities could result in a rhythmic discharge of ganglion cells (after-discharge, cf. Bronk, in this Symposium), because the conditions in the ganglion would correspond to those

postulated by Adrian (1932) for rhythmic discharges initiated by peripheral end organs and injured ends of nerve fibers, as well as to the conditions studied by Arvanitaki (1938 and earlier papers) in crustacean nerves. During the phase of residual negativity significant displacement of ions may be expected (cf. Barron and Matthews, 1938) and, therefore, it is not difficult to imagine that one of concomitant phenomena could be the liberation of acetylcholine and other constituents (cf. Lissák, 1939) of the presynaptic fibers and synaptic knobs. Such liberation of substances, however, would occur after the completion of synaptic transmission by detonator actions and would only lower the threshold of the neuron and facilitate the transmission by detonator negativities of impulses arriving later. Obviously the release of cell constituents would outlast synaptic transmission (delayed output of acetylcholine, 1938*a*; cf. Barsoum, Gaddum and Khayal, 1934).

Electrotonic recording of residual negativity from segment of nerve above block. An interesting phenomenon is revealed by records *6* to *1* in Fig. 23 and *4* to *1* in Fig. 24. The gradient of residual negativity propagates itself not only toward the block, but also backwards toward point *1*, so that it can be picked up by two electrodes on normal nerve, the electrode nearer the block becoming electronegative in relation to the distant electrode. A similar argument is the basis of the method recently suggested by Barron and Matthews (1938 and previous papers) for recording electrotonically through spinal roots potentials developed in the spinal cord during activity; but it follows from the study of the conditions prevailing in the model that this method is not suitable for recording detonator negativities. Close analysis of Fig. 21 would undoubtedly reveal that the front of the spike potential, or even the whole spike, underwent a modification when approaching the block; but these records would not reveal in any other manner the development of the detonator negativity at the margin of the block. Only the existence of residual negativity would show itself in the form of a potential clearly different from that of the preceding spike process. This fact explains why Barron and Matthews (1938) do not describe detonator negativities.* Moreover, electrotonic recording from the roots cannot yield uncomplicated records if the same root is used for recording and leading. This fact is shown by the records in Fig. 23 and 24.

It will be noted in these records that immediately after the end of the train of impulses all the points of the nerve, when pitted against point *11*, *i.e.*, against an unchanged† point of the nerve, appeared to be electronegative. Owing to the spread of the residual negativity accumulated at the

* Even when the electrotonic recording is made with a root adjacent to the root that has conducted the afferent impulses, according to the model, potentials attributable to detonator activities would easily pass unnoticed because detonator negativities are transmitted electrotonically with a great decrement, while the decrement of residual negativities is much smaller.

† "Unchanged" should actually read "as unchanged as it can be." Point *11* was 29 mm. below the center of the block and about 20 mm. above the cut end of the nerve. The demarcation current through it, therefore, was very small at the time of the observation, but it cannot be said that no change at that point had taken place at an earlier time.

margin of the block, the maximal potential was recorded at point *4*, but the distribution of potential along the nerve changed progressively. About 200 msec. after the end of the train positivity was recorded from all points of the nerve and soon afterward, point *1* appeared as that having greatest positivity. If this point had been pitted against point *4* a gradient of negativity would have been recorded because point *4* remained electronegative in relation to point *1* for the whole duration of the potential changes, that took place after the end of the train of impulses; but the recorded gradient between *4* and *1* would not have been a true sign of the changes at the margin of the block. Immediately after the train of impulses, the difference of potential between *4* and *1* was attributable chiefly to electrotonic transmission towards normal nerve of the residual negativity that had accumulated at the margin of the block (Fig. 23, *6*); but later, when this residual negativity changed into residual positivity (cf. the arrow on record *6*, Fig. 23 or the first thin line crossing through records *4* to *10* in Fig. 24), the difference of potential between *4* to *1* must have been caused, to a significant degree, by the process underlying the true positive after-potential of the segment of the nerve (points *3* to *1*) above the block (cf. later).

Residual positivity

The positivity of the nerve above and below the block, when completely developed, had a distribution that was very different from that of the preceding negativity. Immediately after the end of the train of impulses there was an increasing gradient of negativity from point *10* to point *6–4* and a decreasing gradient from point *6–4* to point *1;* but the positivity distributed itself in a different manner because there was only one gradient of progressively increasing positivity from point *10* to point *1*. Examination of the positive troughs of records *1* to *10* in Fig. 24 reveals two important facts: (i) The gradient was not linear, so that at each point of the nerve the amount of positivity developed was in a special ratio to the size of the previous spike or spike-like potentials that was peculiar to each point of the nerve; this ratio being greater below than above the block. (ii) The temporal course of the recorded positivity was different at each point of the nerve. Another important fact is that, while the amount of residual negativity was not an exact indicator of the threshold at the various points of the nerve, pertinent tests showed that the positivity, above as well as below the block, was the sign of a raised threshold.

Impossibility of successful analysis of residual positivity. The model under consideration, on the basis only of the observations thus far described, cannot be used for a direct attack on the problem. A further analysis of the positivity is, in fact, prevented by initial assumptions. The creation of the residual negativity undoubtedly is attributable to certain changes of the polarized membranes of the nerve at the uper margin of the block, but it has been assumed that the transmission of the residual negativity, among other possible processes, was accompanied by a significant displacement of ions. Concentration gradients are by themselves a source of electromotive force, and as soon as they are produced they must establish a current through the input stage of the recording apparatus. This unknown current will algebraically summate with the currents that owe their existence to altera-

tions in the electromotive forces of the polarized membranes of the nerve. Therefore, the recorded potential difference, as it has a complex origin, may not be considered a true sign of any single process in the nerve. Thus, the much abused model, in reaching the limit of its present usefulness, leads to the discussion of an important problem, *i.e.*, that of interpreting potentials recorded with electrodes in contact with a tissue which is the site of processes resulting in the creation of electromotive forces.

Theoretical basis of interpretation of electrical records obtained from nervous tissue

As already indicated, this problem was treated with mathematical rigor by Helmholtz (1853 with review of the early literature) who established several principles that in the course of time have become elementary laws of electrotechnique. If a body within which electromotive forces have developed is placed in contact with an isotropic conducting medium, there is established in the medium a field of current that may be attributed to certain fictitious electromotive surfaces located at the physical boundary of the body and the medium. Where the current leaves the body there appears an electropositive surface, and where the current enters the body, an electronegative surface. Using a more recent form of speech, it may be said, "fictitious sources and fictitious sinks of current." The external field, *i.e.*, the field in the medium, is uniquely determined by the shape and electric properties of the fictitious sources and sinks of electricity (electromotive surfaces). Conversely, the external field, except for an irrelevant constant, uniquely determines the potential at the electromotive surfaces. In consequence, the external field of current is identical with the electrostatic field *in vacuo* between single electric layers with an electric density corresponding to the density of current at the electromotive surfaces. If the body is removed, but the electromotive surfaces are maintained by applying to the boundary of the medium suitable differences of potential that are created in any thinkable manner, the field in the medium will remain unchanged.

Therefore, analysis of the external field alone cannot reveal the nature or position of the true sources of electricity within the body where the electromotive forces are developed,* and an infinite number of true sources of electricity could be assumed that would produce the same external field, *i.e.*, the field measured in the medium. At the time of Helmholtz's writing (1853), knowledge of the structure of muscle and nerve was so incomplete that Helmholtz could make no assumption concerning the true sources of electricity. Still his treatment of the problem on the basis of the principles of mathematical physics led him to the then most remarkable statement that the demarcation currents that had been described for intact muscle could not be compared with the true demarcation current between normal and injured parts of muscle.

The diagrams of Hermann and Cremer. Twenty six years later when ad-

* A lucid presentation of a similar problem is made with elementary mathematical means by Planck (1933, Chapter I and especially Chapter II). Cf. also, MacMillan (1930, with literature), and Kellog (1929, with literature).

vances of histological knowledge justified it, Hermann (1879) presented an illuminating diagram that was reproduced by Cremer (1909) and is reproduced here in Fig. 25, III. Hermann indicated that there were four think-

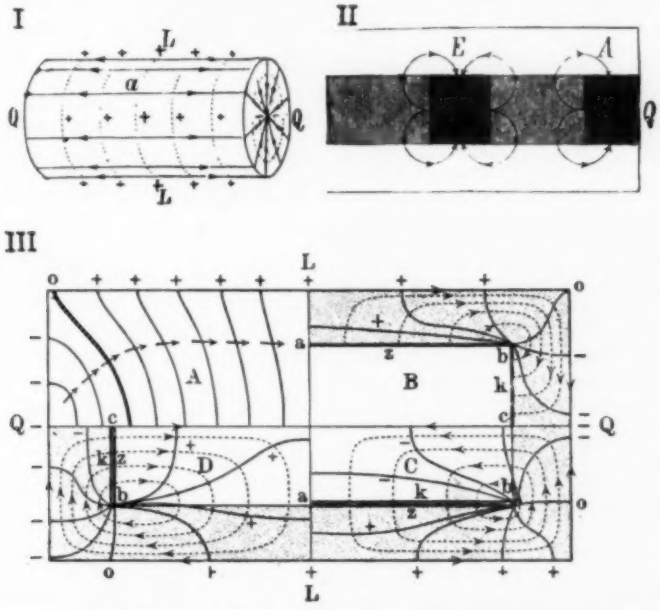

FIG. 25. Diagrams of Herman (1879, Fig. 47, 55, 48) indicating the bioelectric currents observed in muscle and nerve (I and II) and their possible origin (III).

I. In muscle or nerve the longitudinal (uninjured) surface (L) is electropositive in relation to the (injured) cross-sections (Q). Therefore, currents are established in the directions indicated by the arrows.

II. The currents observed in nerve during activity E and after injury A. Q, injured cross section. The injured and the active segment become electronegative in relation to normal or inactive segments because currents are observed in the medium surrounding the fibers, in the direction indicated by the arrows.

III. While diagrams I and II represent observed facts, diagram III represents four "thinkable assumptions" about the electromotive surfaces responsible for the demarcation current. A, a series of transversal electromotive surfaces of the indicated polarity, each reaching the surface of the element; B, an electromotive cylinder positive on the longitudinal section and negative on the cross section; C, double layer, positive outside and negative inside, distributed over the longitudinal section of the cylinder. D, a double layer negative outside and positive inside, located at the (injured) cross section of the cylinder. Hermann indicated that hypotheses A and B are ruled out by the fact that all the points of uninjured muscle are isopotential; between the two remaining assumptions, however, a selection could not be made by studying the external field of current. Hermann favored assumption D (alteration theory of Hermann), but later studies have shown that assumption C is the correct one (membrane theory of Bernstein).

able kinds of true sources of electricity, and although he favored the source illustrated in diagram III, D (Fig. 25), later research led to the admission of diagram III C, which embodies the main concept of the membrane theory. Since at that time the existence theorems of the potential theory, which are usually known under the terms of Dirichlet's and Neumann's problems, had been demonstrated, it became possible, after assuming true sources of

current of known position and constitution, from measurements made in the external field, to reach conclusions concerning changes at the source of current, *i.e.*, the polarized membrane of the nerve fibers.

Thus, Cremer (1899) basing his argument on the mathematical calculations of Weber (cf. Hermann, 1884 and Weber, 1884) assumed that the field of current within a nerve surrounded by a dielectric was comparable to that in a linear conductor, and he was, therefore, able to show how from the density of current outside the nerve fiber, the density of current through the membrane and the change of electromotive force (polarization potential) at the membrane could be calculated by simple mathematical differentiations.

Factors that may complicate the analysis. In the study of the model of synaptic transmission in the phase of positivity a situation has been reached that is more complicated than the simple one considered by Hermann and Cremer. The existence theorems of the potential theory show that unique selection of a potential function among the innumerable functions which may satisfy conditions at a particular zone of the field demands knowledge of the values of the function or of its first derivative, not at any arbitrary surface through the field, but *at the boundaries of the field*. Given this boundary condition, the problem admits of one and only one solution; but if that condition is not given, an infinite number of solutions may be found, all of them having in common the values of the function at the fictitious electromotive surfaces of Helmholtz, *i.e.* at the surfaces of contact of the recording electrodes with the tissue.

Consequently, so long as it is assumed that the currents in the medium surrounding the active neuron are attributable exclusively to changes in the electromotive force in different parts of the membrane of the neuron, the records may safely be interpreted in the form that has been suggested while discussing Fig. 14 and 17 of this report. But if it be admitted that in the tissues other sources of electromotive force may develop, then the records do not admit of a unique interpretation. For phenomena of short duration, such as have been considered in this paper, it seems reasonable to assume that the main sources of potential are alterations of the electromotive force of the polarized membrane of somas and axons of nerve cells; but for long-lasting phenomena, which may involve chemical and concentration changes, the situation is different and a most careful analysis must be made before reaching a conclusion. The problem, however, does not seem as hopeless as it was in Helmholtz's time because, after all, the number of kinds of sources of electromotive force that may appear in nervous tissue is limited, and it may be hoped that careful mapping of the electric field in the brain tissue, in and around the active zone, will ultimately yield sufficient information to differentiate the various true electrical sources and investigate the changes they have undergone. In the case of the model of the synapse that has been subjected to study in this report, the available records measure only potential differences at the boundary of nerve and dielectric, but do not give any information about the internal field of the nerve trunk. Since the assumption has been made that during the transmission of the gradient of residual negativity, significant displacement of ions takes place, a knowledge of the field within the nerve trunk is necessary in order to ascertain how far the positivity is attributable to changes in the polarization of the membrane and how far to other electromotive sources.

Definition of true after-potentials

One more remark must be made before closing the analysis of the synaptic model, namely, that the analysis, besides other defects, has the great one of not having considered true "after-potentials." There was, however, a good reason for the omission. In the model, owing to the existence of the block, great potential gradients developed, and these seriously interfered with the study of after-potentials. Potentials that simulated after-potentials were observed, but they were not true after-potentials.

In agreement with the thesis developed by Gasser (1937 *a*, *b*) on the basis

of a considerable body of evidence, true after-potentials may be characterized as follows: After-potentials develop in nerve fibers following the conduction of spike processes, and their slow time course prevents their causing measurable potential gradients in normal nerve, because the entire length of nerve available for experimentation during the after-potential stays "in phase." Thus, after-potentials can be measured only by pitting points of the nerve that have been active against a killed end of the nerve separated from the live nerve by a sharp line of demarcation.

According to this definition the processes underlying the after-potentials are measured by their electric signs. But while the spike potential, besides being the sign of a process, is a stimulating agent, the after-potentials are purely signs of a "process of unknown nature, which affects the condition of the plasma membrane" (Gasser, 1937*a*, p. 167). Therefore true after-potentials, since they have been defined purely as signs of processes that among other things determine the course of recovery after activity, should not be directly homologized with potentials that act as agents. This statement perhaps needs clarification by an example. A nerve acquires supernormal excitability after conduction because its membrane during restoration undergoes a certain process, the sign of which is the negative after-potential; but it is not supernormal, because the negative after-potential is stimulating it subliminally. On the other hand, the segment of nerve below the cocaine block acquires a lowered threshold when the residual negativity gradient at the upper margin of the block acts upon it as a stimulus. This potential when recorded looks like a negative after-potential, but in a strict sense it is not purely the sign of changes that may be happening in the membranes of the nerve below the block and must be in part a propagated electric wave.

For these reasons the writer has carefully avoided the use of the term "after-potential" when describing the recorded differences of potential which, it is true, mimic after-potentials, but hardly can be considered as pure signs of the processes taking place when true after-potentials are recorded. Obviously, as the number of elementary processes that can take place in the polarized membranes of nerve fibers is limited, future research undoubtedly will establish relationships between the processes underlying the cumulative residual negativity and the following positivity, and the processes that take place in nerve after conduction to produce the negative and positive after-potentials. Perhaps it will become possible, when knowledge is sufficiently advanced, to use the same terminology for all processes that have similar electric signs, but until then it seems more advisable to use different terms for concepts as different as the negative after-potential, which is defined as a sign, and cumulative negativity, which has been considered in part a stimulating agent.

IV. Concluding Remarks

It is hardly necessary to emphasize that the preceding analysis of the cocaine block has consisted of a study of experimental facts, insofar as the

processes in the treated nerve were concerned, but that it became a theoretical argument whenever comparisons were made with processes happening at the motoneuron and its synapses. Nevertheless, it may be considered a useful analysis, because it has led to the examination of significant questions and has offered a statement of the electrical theory of synaptic transmission in a form that is suitable for detailed discussion and is capable of suggesting new experiments. The electrical theory, as presented in the light of the model, bridges a number of the differences in the views of recent investigators, which although in remarkable agreement in the interpretation of some phenomena, are in disagreement in the interpretations of other points. For example, the assumption of the production of two successive gradients of negativity of the synaptic knobs, if it were generally admitted, would unify the views of Adrian, Barron, Bronk, Bremer, Eccles, Gasser, Lorente de Nó, Matthews, and Sherrington. Detonator negativities with a temporal course similar to that of the spike process of the nerve impulse would cause a strong brief excitation; internuncial bombardment would insure restimulation by detonator actions and temporal summation. Residual negativities would also enter into the production of temporal summation. Moreover, residual negativities and, eventually, residual positivities, not only would be effective in producing changes in threshold, but would also result in environmental changes of the type demonstrated by Dusser de Barenne, McCulloch and Nims (1937). After the masterful analysis of Dusser de Barenne and McCulloch (1939) it is unnecessary to insist upon the harmonious blending of all the factors that take place during activity of the nervous system.

The model has perhaps done even more; it has emphatically shown that —as Gasser has indicated in the Introduction to this Symposium, and Erlanger and Bronk have pointed out in their contributions—the investigation in peripheral nerve of the properties of nervous tissue is a direct and profitable method of study of the elementary processes that take place in the nervous system. Knowledge of the properties of nerve may not result in the immediate solution of the problems offered by the central nervous system, but it supplies challenging analogies and working hypotheses.

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PROBLEMS OF SYNAPTIC FUNCTION

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THE GREATEST achievements in science have been those which brought unity out of diversity. Observation reveals complexity and diversity everywhere. The great milestones are the discoveries of common trends or laws running through the complex pattern, or simple units of which it is built. Thus, while the biochemist has been giving us increasing molecular weights for the proteins, running now into millions, the atomic physicist has been reducing all substances to 3 or 4 units—proton, electron, neutron, etc. The animal body obviously possesses vast complexity, and no other system in it is so complex in structure or behavior as the nervous system. Twenty-five years ago, Keith Lucas (1917), after uncovering much of the nature of the functional response in nerve-fiber, faced the formidable array of functions which had been ascribed to nerve-centers with the question:

> "Are we to suppose that the central nervous system uses some process different from that which is the basis of conduction in peripheral nerves, or is it more probable that the apparent differences rest only on our ignorance of the elementary facts of the conduction process? If we had a fuller knowledge of conduction as it occurs in peripheral nerve, should we not see Inhibition, Summation, and After-discharge as the natural and inevitable consequences of that one conduction process working under conditions of varying complexity?"

His answer to his own question was the suggestion

> "that we should inquire first with all care whether the elementary phenomena of conduction, as they are to be seen in the simple motor nerve and muscle, can give a satisfactory basis for the understanding of central phenomena; if they cannot, and in that case only, we shall be forced to postulate some new process peculiar to the central nervous system."

This suggestion has found an echo more than once in physiological thinking since it was uttered—most recently in a schema offered by Gasser (1937 a, b).

Histology forces us to accept complexity of structure. There are many different kinds of synapse. Each afferent neuron divides into many end-branches. Histologists have recently estimated as many as 1300 end-bulbs on a single anterior horn cell. There is evidence that in general more than a single impulse in a single afferent axon is required to evoke a reflex response. Granting this, obviously even if each terminal branch brings to the synapse an impulse of all-or-none character, there is room in the above number of end-bulbs leading to the final common path to provide for a vast amount of gradation in the central excitation effect.

A recent monograph (Fulton, 1938) has said, "The simplest reaction of the nervous system is the segmental spinal reflex involving two elements, an afferent neuron and a motor unit." There is a temptation to simplify the picture thus for the portrayal of the sequence of events. But it is an oversimplification, which may be misleading since the most salient feature

of a reflex center seems to be branching and multiple interconnection, such that one afferent fiber makes many central connections and one motor neuron receives impulses from many converging paths. In a telephone system involving only two subscribers a central exchange would be superfluous; a single wire would suffice. To schematize the reflex arc as consisting of but two units is to miss the essential distributing property of the center.

Granting the high degree of structural complexity, which the pictures just shown by Dr. Lorente de Nó have emphasized anew, we face the question,—can we simplify the terms of the problem physiologically by finding any unity of function throughout the system? There is a highly significant unity in the conduction process common to nerve and skeletal muscle. In spite of the structural and functional differences between these tissues, all the essential properties in their mode of conducting the propagated disturbance are the same. This appears in the similarity of their excitation by electric currents. It is more strikingly revealed in the refractory phase and the all-or-none character of response—features which Adrian (1914) has shown to be causally connected, and which are both common to nerve and skeletal muscle. Finally, the similarity of the electric response or action potential is so close in the two tissues as to leave little doubt of a fundamental kinship in the underlying activities. Indeed, Lapicque (1926) has shown that the nature of electrical excitation, as attested by the curves relating strength and duration of the current required to excite, is essentially the same in a large variety of excitable tissues. Electric responses, in which the active region is negative with respect to inactive tissue, are also common to various excitable tissues of animals, including others besides nerve and striated muscle,—e.g., smooth muscle, gland and probably nerve-cells. This property, then, seems to be common to all animal tissues capable of functional response.

There was further admirable simplification when Erlanger and Blair (1931a, b) identified refractory phase in nerve with the electrotonic effect of current-flow—post-cathodal depression. A further step in the same direction is the evidence that, in nerve, facilitation is correlated with negative after-potential, and depression of excitability with positive after-potential (Erlanger and Gasser, 1937). Neatly correlated with that is the evidence of Hughes, McCouch, and Stewart (1937) that in the spinal cord inhibition is coincident with positive after-potential.

In the cervical sympathetic ganglion Eccles (1935a) reported a corresponding association of facilitation with negative after-potential, and inhibition with positive after-potential. But Rosenblueth and Simeone (1938a) in a thorough investigation were unable to substantiate these correlations. Their evidence pointed to the existence of other factors.

Sherrington (1925) emphasized the indications that in the center there is some process which differs from the nerve impulse in having no refractory phase and which instead is capable of accumulation by summation and thus of graded intensity. This hypothetical process he designated "central excitatory state" (c.e.s.), remaining noncommittal as to its nature. It might be

a substance whose concentration could be varied, or it might be the degree of depolarization of a membrane, polarized in its resting state. A substance is more easily visualized, and in the elaboration of Sherrington's idea it was designated by Fulton (1926) a "chemical theory." But "c.e.s." is still referred to in the noncommittal sense (Eccles, 1937a).

Chemical mediation of the effects of nerve impulses was first established in the case of vagus inhibition of the heart by the observations of Loewi (1921). Similar chemical mediation seems to be well established in the case of the activation of smooth muscle by the nerve impulse. In the case of skeletal muscle, the question is more controversial, but there is an impressive accumulation of evidence pointing to chemical mediation at the neuromyal junction.

In the sympathetic ganglia, especially the cervical, a great mass of evidence has been adduced to show the liberation of acetylcholine (ACh) in sufficient quantities to excite the ganglion cells (Kibjakow, 1933; Feldberg and Gaddum, 1933, 1934; Feldberg and Vartiainen, 1934), and the inference has been drawn that this substance is the synaptic transmitter (cf. Dale, 1934, 1935; Rosenblueth and Simeone, 1938a, b). The evidence in support of chemical mediation in the synapses of the central nervous system is not so clear, but if it is finally established that intercellular transmission is chemically mediated in such widely different systems as the neuromuscular junctions of smooth and striated muscle and the synapses of ganglia, the operation of a wholly different mechanism in the histologically similar synapses of the central gray matter would be a most surprising anomaly.

In spite of the strong evidence and arguments for chemical mediation, its claim to recognition as the synaptic transmitter has been seriously challenged. Much of the controversy has centered around cholinesterase, which exists in the tissues and has the property of destroying ACh by hydrolysis. Eccles (1937a, b) conceded that ACh increases the excitability of ganglion cells, but contended that cholinesterase could not destroy the ACh quickly enough to account for the rapid decay of the "synaptic transmitter" indicated by his observations. He reinforced this argument (1937a) by the failure of eserine (which protects ACh from cholinesterase) to prolong the action of the transmitter in his experiments. Rosenblueth and Simeone (1938b) found that with larger doses of eserine the decline of the transmitter (or mediator) is measurably retarded, and thus they removed one objection to the chemical theory. That the rapid decay of the transmitter may not be a serious obstacle will appear in view of other considerations to be mentioned presently.

The central excitatory state has been likened to the local excitatory process which Lucas (1917) postulated as the essential preliminary to setting up a propagated disturbance in nerve or muscle (Creed *et al.*, 1932, p. 45). The resemblance rests chiefly on the fact that in each case there is evidence of gradation of the excitatory tendency and, by virtue of this gradation, of the cumulative effect of summation. In each case it appears that when the

of a reflex center seems to be branching and multiple interconnection, such that one afferent fiber makes many central connections and one motor neuron receives impulses from many converging paths. In a telephone system involving only two subscribers a central exchange would be superfluous; a single wire would suffice. To schematize the reflex arc as consisting of but two units is to miss the essential distributing property of the center.

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excitatory state, or process, reaches a certain requisite intensity, it causes the discharge of an impulse. Certain evidence has been taken to signify that the central excitatory state (what Eccles now calls the "detonator action") disappears when it has reached threshold value and set up a discharge (Creed *et al.*, 1932, p. 44), or at least that "its subsequent course is submerged by the consequent refractory period" (Eccles, 1937a, p. 21). The implication is that it can never become supraliminal. Objection has been raised to the chemical theory on the ground that acetylcholine in large quantities can cause a continued repetitive discharge of motor neurons, which seems to be evidence of its persistence in supraliminal quantity and thus to render impossible its identification with the rapidly decaying synaptic transmitter. But it is quite possible that small quantities of ACh, sharply localized, can very quickly fall below threshold concentration, while a larger and more widespread production of it would make possible a persistent, supraliminal concentration. In this connection it has previously been suggested (Forbes, 1934) that a chemical mediator might be elaborated in a reservoir in which its quantity could vary according to the number of excitatory impulses producing it, yet at the point where it initiates the outgoing nerve impulses, it might never reach a supraliminal concentration, because in some way it would expend itself in the act of initiating a discharge. The reservoir might be the synaptic end-bulbs or the dendrites or the perikaryon. The point of initiating the outgoing nerve impulse might be the cell-membrane or the axon hillock.

When it comes to localizing events whose existence can only be inferred great caution is indicated. The argument of Eccles and Hoff (1932) that antidromic impulses pass backward over the cells has been summarized by Gasser as follows: "In their experiments, rhythmically discharging neurones have their rhythms altered by a volley back-fired into the cord; and an alteration in rhythm such as this would hardly be possible if the impulse did not get back to the point at which excitation of the neurone takes place" (Erlanger and Gasser, 1937, p. 189). It by no means follows from this evidence that the antidromic impulse traverses the entire neuron to its dendrites. Collision with the disturbance at various possible points within the cell might well cause interference. All we can safely conclude is that the antidromic impulse reaches the point at which the discharge is initiated, be it synapse, dendrite or axon hillock.

Concerning the disappearance of acetylcholine, even if its only means of dissipation is destruction by cholinesterase, the recent observations of Feng and Ting (1938) and the independent observations of Marnay and Nachmansohn (1937) have shown that in skeletal muscle cholinesterase is far more concentrated in the vicinity of the neuromuscular junction than it is in the rest of the muscle or than has been previously supposed. Feng and Ting conclude that its concentration is adequate for the rapid destruction of acetylcholine required by the chemical theory of transmission. These observations deal only with the neuromuscular junction, but there seems to be no reason why similar high concentration should not exist at the synapse

between neurons, where so many other features of intercellular transmission run a parallel course to that which has been found in the neuromyal junction.

In support of the electrical theory of synaptic transmission, Erlanger has just shown us that, in a nerve-fiber in which one or two internodal segments amounting to 1 or 2 mm. in length are blocked by anodal polarization, the action potential may restimulate the fiber beyond the inactive segment. In a further report on these observations Blair and Erlanger contend that, if the action potential can thus restimulate the axon across an inactive stretch of 1 to 2 mm., it is justifiable to conclude that it can excite the tissue beyond a synapse, "and that it will unless the synapse includes a device for preventing current spread" (1939, p. 105). In answer to this contention, it should be emphasized that the structural and presumably the electrical conditions are quite different in an unbroken but inactive axon from those at the synapse. Here, at the termination of the neuron, histology seems to reveal a transverse membrane, which may well act as a short-circuit to the action potential which is responsible for the effect observed by Blair and Erlanger. If the membrane theory of nerve conduction holds good, there is every reason to expect such a short-circuiting effect at the termination of the axon. Indeed, this anatomical consideration is perhaps one of the strongest reasons for seeking a different mechanism, such as chemical mediation, as an essential step in the excitation of the next neuron.

Lorente de Nó (see Forbes, 1936, discussion) has shown that a subliminal induction shock applied directly to a motor neuron and a subliminal afferent volley impinging on the same motor neuron can sum to produce excitation. He argues from this that the two exciting agents must be alike in kind and that therefore synaptic transmission is due to electrical excitation by the action potential. Yet, why should not a chemical substance which has the power of depolarizing the cell-membrane sum with an electric current which also depolarizes the same membrane? The evidence of community of effect is suggestive of a common cause, but not conclusive.

Rosenblueth and Morison (1937) in an investigation of the neuromyal junction of skeletal muscle showed that the effect of curare, eserine, and fatigue, and the phenomena of Wedensky inhibition, all present difficulties in the way of explaining transmission on the electrical theory, but are readily explained on the basis of mediation by acetylcholine, without resort to assumptions which have not been supported by experimental evidence. Cannon and Rosenblueth (1937) found essentially similar results in their observations on the cervical sympathetic ganglion, which strongly reinforce the chemical theory as applied to the synapse between neurons.

So goes the controversy. Dale in discussing it remarked that it was unreasonable to suppose that nature would provide for the liberation in the ganglion of acetylcholine, the most powerful known stimulant of ganglion cells, for the sole purpose of fooling physiologists. To this Monnier replied that it was likewise unreasonable to suppose action potentials would be delivered at the synapses with voltages apparently adequate for exciting the ganglion cells merely to fool physiologists. The consideration of the probable

short-circuiting effect of the termination of the neuron, mentioned above, appears to weaken the electrical argument and to strengthen the case for the chemical theory.

A review of this controversy between the electrical and the chemical theories of synaptic transmission recalls the experience of physicists in the study of light. When they perform an experiment designed to show that light is a wave-action, the experiment yields an affirmative answer and shows what it was intended to. When they perform an experiment designed to show the corpuscular nature of light, it also yields an affirmative answer. These results appear to present a direct contradiction, yet, for some reason which is quite beyond my powers of comprehension, the physicists seem perfectly happy about it and maintain that there is no real contradiction. I wonder if the electrical and chemical theories of synaptic conduction may also prove not to be mutually contradictory after all. Bronk (1939) has furnished evidence which leads him to a pluralistic view of synaptic function. Conceivably, we are dealing with electro-chemical events which have both electrical and chemical aspects, revealed according to the nature of the experiment. Every chemical effect is bound to involve a potential change and electrical forces in any system of chemical substances in solution must tend to produce motion of charged ions. There is evidence that potassium is liberated from a nerve when it conducts an impulse. Lissák (1939) has recently furnished evidence of the liberation of acetylcholine in active nerve-trunks. Brown and Feldberg (1936) showed that potassium chloride may stimulate ganglion cells and may also liberate acetylcholine. These observations suggest that we are dealing with a chain of electro-chemical events both in the axon and at the synapse and that the differences between them may possibly be differences in degree, as, for example, differences in the quantity of chemical substance liberated or in the distance it may migrate in different parts of the nervous structure. It has been suggested (Rosenblueth, personal communication) that the synaptic transmitter ("detonator action," according to Eccles) is acetylcholine, and that the slower and more prolonged effect which Eccles now designates "c.e.s." may represent a secondary change in the equilibrium of potassium ions in the system.

Much of the discussion has been based on the membrane theory of nerve conduction. It has already been pointed out (Forbes, 1936) that no anatomical structure in nerve has been identified with the supposed membrane, and that the observations of Cohn (1935) on the dipole moments of long protein molecules may perhaps give a clue to a more adequate theory of nerve function. Disturbances in the equilibrium of such molecules might conceivably produce effects which simulate the depolarization of a membrane. The place which acetylcholine might occupy in such a system seems to offer an interesting field for future research.

Summing up this question of the relation between the chemical and electrical events in the synapse, we might look for a sequence of events which, to use Lloyd Morgan's phrase (1901), presents a "dualism of aspect, distinguishable in thought but indissoluble in existence." But we must

not think so loosely as to overlook the fact that the electrical potential which excites a tissue is the same thing whether produced by a dynamo or by a galvanic cell, and this is quite distinct from acetylcholine,—a substance which can be put in a bottle, whatever its electrical properties.

In conclusion, I may cite the observations reported last year by Renshaw, Forbes, and Drury (1938) in which micro-electrodes inserted into the pyramidal cell layer of the cat's hippocampus revealed two wholly distinct types of electric response. Smooth-contoured slow waves were derived from all parts of the hippocampus, whether the exploring electrode was in the axon layer, the cell layer, or the dendrite layer. When the micro-electrode was in close proximity to the cells, and only in that region, a wholly different type of discharge was also found. This consisted in monophasic spike potentials of approximately the same duration as axon potentials in the A fibers. Usually they appeared in groups of four or five, with progressively declining voltage and frequency, the whole group lasting only 15 or 20 msec. That these two types of discharge are wholly distinct phenomena is shown by the fact that no intermediate forms were found in any of our experiments; no correlation was seen between them. The time-relations of the quick responses suggest the discharge of impulses from the cell-body. The fact that the micro-electrode adjacent to the cell always became negative during these rapid spikes suggests localized depolarization or negativity over the surface of the cell-body, or a part of it, with reference to the more widely distributed portion of the cell, *e.g.*, the dendrite.

The chemical and anatomical complexity of the nervous system should warn us to beware of adopting too readily alluring schemata with any confidence that they really will embody the truth about nervous function. It will be necessary to keep an open mind about various theories for a long time to come. As yet, we have hardly any clue to the switching mechanism whereby such things as volition are achieved, that is, the way in which one path or another may be open to the streams of impulses under the varying conditions which occur in the life of animals.

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